

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
24 June 2004 (24.06.2004)

PCT

(10) International Publication Number
WO 2004/053099 A2

(51) International Patent Classification⁷: C12N

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(21) International Application Number:
PCT/US2003/039499

(22) International Filing Date:
10 December 2003 (10.12.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/432,496 10 December 2002 (10.12.2002) US

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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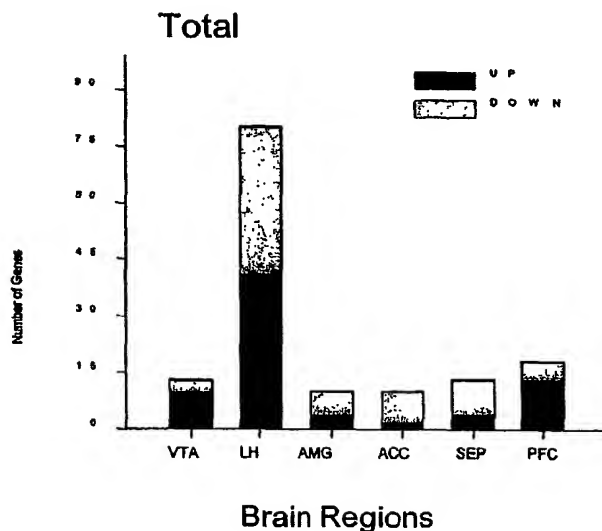
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Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: A METHOD FOR TREATMENT OF DRUG ADDICTION AND FOR SCREENING OF PHARMACEUTICAL AGENTS THEREFOR



(57) Abstract: The present invention is directed to a method for treatment of drug addiction and screening methods for identifying pharmaceutical agents that ameliorate or prevent the deleterious effects of addiction. The invention is as well directed to a group of genes and a group of gene products that are up or down requested as a result of addiction.

WO 2004/053099 A2

**A METHOD FOR TREATMENT OF DRUG ADDICTION
AND FOR SCREENING OF PHARMACEUTICAL AGENTS
THEREFOR**

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Government Funding

The invention described herein was developed with support from the National Institute on Drug Abuse (NIDA) under Grant Number DA 13821. The U.S. Government has certain rights in the invention.

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Background of the Invention

A current challenge for the neuroscience of drug addiction is to understand the molecular mechanisms responsible for the development of compulsive drug use (Koob et al., 1998). Such a transition is generally associated with a pattern of escalating drug use whereby consumption increases over time and becomes more and more difficult to control. This pattern often leads to antisocial behavior, physiological addiction, physical debilitation, contraction of disease and ultimately, death.

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Social scientists, behavioral scientists and biological researchers have devoted significant efforts toward ameliorating the deleterious effects of this pattern. Use of hospitalization, counseling, treatment programs and withdrawal management has been part of continuing attempts by society to minimize addiction.

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Scientists have also studied the physiological changes associated with drug addiction. They have established that the body's metabolic pathways undergo significant alteration during drug addiction. In particular, these alterations make withdrawal painful and re-addiction attractive. Although the direct biochemical interactions of such opioid drugs as morphine, heroin and cocaine have been elucidated, the upstream and downstream biological effects of these interactions have not. For example, the three kinds of opioid receptors mu, delta and kappa, and the dopamine receptors are well-known as the primary receptor sites for opioid interaction. Nevertheless, how the activation of these

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receptors affects upstream and downstream pathways in tissues such as the central nervous system is unknown.

One of the problems facing research scientists investigating drug addiction has been the lack of an animal model that tracks the escalating need present in humans. The known animal models typically involve plateauing consumption and effect of opioid intake. The physiological consequences of the plateau prevent the identification of genes that are up and down regulated as a result of the increasing dependency and physiological need for the opioids. In fact, few major changes in protein expression were found in the past to be related to cocaine addiction. Because of this failure, researchers have been unable to predict or correlate genetic consequences and drug dependency.

Therefore, there is a need to develop an assay to determine the up and down regulation of genes during escalating drug addiction. A further need is the identification of sets of up and down regulated genes that can be used as screens for pharmaceutical agents helpful in the treatment and/or ameloration of the causes and consequences of drug addiction. Yet another need is the identification of pharmaceutical agents that will treat the deleterious effects of addiction. A still further need is the therapeutic use of pharmaceutical agents for treatment of drug addiction where the agents do not interact with the primary opioid and dopamine receptors involved in opioid drug response.

Summary of the Invention

These and other needs are met by the present invention, which is directed to a method for treating drug addiction, especially opioid drug addiction. The invention as well is directed to a method for screening for pharmaceutical agents useful in such treatment. The invention is also directed to a set of mammalian genes that are up or down regulated during escalating drug use and to a set of corresponding gene expression products.

The treatment method according to the present invention involves administering to a patient in need of such treatment one or more pharmaceutical agents that interact with the genes which are up or down regulated during the course of escalating drug use, or that interact with the corresponding expression products, or that interact with the targets of such expression products, such as receptors. Hence, a beneficial interaction of the pharmaceutical agent is an

interaction that ameliorates, blocks or prevents the abnormal up and/or down regulation of these specifically identified genes, or is an agonist, antagonist, inhibitor, activator, blocker mimic or anti-mimic of the expression product or its target.

5 The screening method according to the present invention involves use of an *in vivo* or *in vitro* screen to identify one or more pharmaceutical agents that interact with the expression products of genes which are up or down regulated during escalating drug use or which interact with the targets of such expression products, such as receptors.

10 The invention as well is directed to a set of mammalian genes and a set of their expression products that are uniquely up or down regulated during escalating opiate use. The set of genes includes those that encode certain signaling molecules or ligands, certain enzymes, certain ion channels, certain receptors, certain cytoplasmic receptor coupling proteins, certain transmembrane
15 molecular transporters, certain ESTs and certain growth, survival, functional or structural (gsfs) proteins. In particular, these genes encode the following proteins:

- 20 A) Signaling molecules (ligands) which include insulin-like growth factor II, interleukin-3 (IL-3), interleukin-3 beta, fractalkine/chemokine CX3C motif ligand 1, platelet derived growth factor A chain, Neuroligin 3, neuron-specific protein (PEP-19), Synaptamin XI;
- 25 B) Enzymes which include catechol-O-methyltransferase, beta-andrenergic receptor kinase, Ras-related GTPase, Ras-related GTPase beta S-100, aromatic L-aminoacid decarboxylase, beta andrenergic receptor kinase, Synaptagmin III, and G-protein beta-1 subunit;
- 30 C) Ion channels which include potassium channel beta subunits, sodium channel beta 2 subunit, voltage gated potassium channel Kv3.4, Saw-related subfamily member 2, potassium channel delayed rectifier, potassium inward rectifier 10 (Kir 4.1), and calcium channel alpha 1 subunit;
- D) Receptors which include AMPA receptor GluR1, Kainate receptor KA1, Peripheral benzodiazepine receptor, alpha 2-

- adrenergic receptor, NMDA receptor-like complex glutamate binding protein, GABBA receptor alpha 3 subunit, tumor necrosis factor receptor chain (p60), NMDA receptor subunit 2D, and non-processes neurexin1-beta mRNA;
- 5 E) Receptor coupling proteins;
- F) Transporters which include vesicular inhibitory amino acid transporter and sodium dependent high affinity glutamate transporter, sodium or potassium ion transporting ATPase alpha 2 subunit,
- 10 G) ESTs which include AA799879 and AA956149, (genes);
- H) Growth, survival, functional, structural proteins which include Bcl-x alpha, signal transducer and activator of transcription 3 (STAT3), Retinoblastoma protein, Nsyndecan (syndecan-3 or Neuroglycan), EST189376, Synaptotagmin VIII, Calcium ion
- 15 binding protein, and Microtubule-associated protein (MAP1A).

Particularly provided are genes encoding Platelet-derived growth factor A chain; Neuroglycan; Neuroligin 3; Na⁺,K⁺-transporting ATPase alpha 2 subunit; Na⁺,K⁺-ATPase beta 2 subunit; and NMDA receptor subunit 2.

20 The treatment method according to the present invention may be accomplished by administration of an effective amount any one or combination of the following:

- 1) an agonist or antagonist of a receptor of group D or a receptor that is a target of the foregoing group of signaling molecules, group A, including, but not limited to, NBQX, CNQX, LY300168, GYKI53655, 3-CBW, matrix metalloproteases (MMP), tyrophostin AG 1024, AG1295, AG-1296 and the GABA agonist Gabapentin,
- 25 2) a mimic or anti-mimic of a signaling molecule (ligand) of foregoing group A wherein the mimic provides a similar three dimensional configuration and electronic interaction as the signaling molecule or ligand and the antimimic is the opposite, i.e., prevents binding with the corresponding target,
- 30 3) an anti-signaling molecule or anti-ligand corresponding to the foregoing group A wherein the anti-signaling or anti-ligand binds to,

interferes with, or alters, such as by cleavage, the signaling molecule (ligand), including, but not limited to, matrix metalloproteases (MMP), tyrphostin, tyrphostin AG490 and batimastat,

- 4) an activator or inhibitor of an enzyme of foregoing group B,
- 5 5) a blocker or activator of an ion channel of foregoing group C, including, but not limited to, barium, TEA, 4AP, BDS and calphostin C,
- 6) an activator, inhibitor, agonist or antagonist of a receptor coupled protein of foregoing group E,
- 10 7) an activator or inhibitor of a transporter of foregoing group F,
- 8) an activator or inhibitor of an EST of foregoing group G,
- 9) an activator or inhibitor of a growth, survival, functional, structural protein of foregoing group H, including, but not limited to, tyrphostin AG490, Ghrelin, NPB/NPW, AGRP, NPY, MCH, Orexyn A/B,
- 15 galanin/GALP, Beacon, beta-endorphin, dynorphin, GHRF, alpha-MSH, CART, PYY3-36, NPB, CRF, urocortin II, III, GLP-I, oxytocin, neurotensin, CCK, GRP, bombinakinin-GAP, neuromedin, POMC, ADM, somatostatin, TRH, and CGRP.

The pharmaceutical agent effective for treatment according to the
 20 invention may be administered as a pharmaceutical composition of a pharmaceutical agent and a pharmaceutical carrier. The carrier is chosen according to the dictates of the route of administration.

The method for screening according to the invention may be accomplished by *in vivo* or *in vitro* techniques. The *in vivo* technique involves
 25 use of an animal model and either a historical or current positive control wherein the test animals are treated with an increasing dosage of addicting drug and before, simultaneous with, or after beginning the addicting drug administration, are given the potential pharmaceutical agent. mRNAs from specified brain sections of the test animals can be obtained sequentially and screened in a multi-
 30 well assay to determine up and down regulation of the genes mentioned above. A lessening of the up and/or down regulation of one or more of these genes relative to the historical or current positive control indicates that the potential pharmaceutical agent will be useful in the treatment of drug addiction.

The method for screening according to the invention may also be accomplished by an *in vitro* technique. Cells may be contacted with a potential pharmaceutical agent and mRNA may be extracted from the cells. The mRNAs can be screened to determine if the potential pharmaceutical agent caused an increase or decrease in the expression of the gene products described herein as associated with drug addiction. Gene expression may also be determined through use of other known biological assays that include radioimmunoassay, ELISA, southern blot, northern blot, enzymatic activity and the like to establish whether or not appropriate activity is present.

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Brief Description of the Drawings

Figure 1.a) Escalation in intravenous cocaine consumption in rats. Mean (\pm s.e.m.) number of intravenous cocaine self-injections obtained during the first hour of each daily session of cocaine self-administration. (* different from ShA rats, $p < 0.05$, tests of simple main effects after appropriate two-way analyses of variance).

Figure 1.b) Total number of probe sets per brain region that significantly change by more than 1.8-fold in LgA (long access) rats compared to control levels measured in drug-naïve rats. (c) Fraction of total probe sets that significantly change in LgA rats compared to both ShA (short access) and drug-naïve rats (ES genes). *Abbreviations:* VTA, ventral tegmental area; LH, lateral hypothalamic area; AMG, amygaloid complex; ACC, nucleus accumbens; SEP, septal area; PFC, medial prefrontal cortex.

Figure 2. Correlation between changes in gene expression levels in rats with differential access to intravenous cocaine self-administration (see Methods). In both groups, the expression level corresponding to each probe set was normalized to the control level measured in drug-naïve rats (see Methods for details). Normalized values range from 0 to 1, with 0.5 corresponding to no change from the control level. The central square in each graph contains all probe sets that do not change by more than 1.8-fold in both ShA rats and LgA rats (see Methods for details). Each point represent a single gene (over 1300 probe sets) and each graph represents a different reward-related region of the brain (6 in total).

Detailed Description of the Invention

The present invention is based upon an animal model for drug addiction that more accurately tracks the course of drug addiction in man. While traditional models limit access to the addicting drug, this model enables ever-increasing dosing if desired by the test animal. In this model, drug intake gradually escalates over time when daily access to the drug is increased to 6 or more hours (Ahmed et al., 2000; Ahmed and Koob, 1998). Using this model, genes specifically associated with drug addiction in selected reward-related brain regions have been identified.

10 The opiate, cocaine, was the drug of choice used in the study. This drug displays a typical opioid addiction pattern and will predict the behavior and physiological reaction of the group of opioid drugs. It is known to interact with the opioid and dopamine receptors of the central nervous system of mammals. However, the methods of the invention may also be used in association with
15 other addictive substances.

Thus, the present invention specifically investigates escalation of cocaine intake, which a) is a superior model for drug addiction and b) selects from the large number of altered transcripts in the transcriptional profilings only those mRNAs and gene products which themselves, or the ligands thereof, could be
20 used to treat human drug addiction.

According to the invention, the raw experimental evidence shows that a large number of genes are responsive to cocaine self-administration (self-administration-associated genes, SA genes). However, when the results using the traditional model and the new model of administration are compared, only a
25 small fraction of those genes changed their expression specifically in association with escalation of cocaine intake (escalation-associated genes, ES genes). Of all the brain regions examined, the lateral hypothalamus area was the most genetically responsive. The pattern of ES genes observed within this area indicates that compulsive drug use is associated with a profound remodeling of
30 lateral hypothalamic intrinsic circuitry involving glutamatergic neurotransmission. Many of the ES genes identified are also expressed during development and/or are involved in neural plastic processes in the adult brain, such as neurogenesis, synaptogenesis, regulation of synaptic strength and responses to neurotoxic stress. It is believed that these results indicate that brain

reward pathways undergo a large-scale reorganization, both structurally and functionally, during the transition to drug addiction. These neuroadaptive changes contribute to the chronic deficit in reward function recently reported after cocaine intake escalation (Ahmed et al., 2002).

5 Accordingly, the invention concerns the identification of gene targets in the escalating addiction animal model that have already interacted, or will interact, with the addicting drug. Identification of these up and down regulated genes of the animal model and their correlation with corresponding human genes predicts physiological changes occurring in human addiction. The identification
10 also enables significant advances in treatment of addiction.

 According to the invention, the identified gene targets include the following.

 A) Genes encoding signaling molecules that include Insulin-like growth factor II, interleukin-3 (IL-3), interleukin-3 beta,
15 fractalkine/chemokine Cx3 C motif ligand, neuroligin 3, PDGF, neuron-specific protein (PEP-19), and Synaptamin XI;

 These signaling molecules, and the agonists and antagonists for their corresponding receptors, as well as mimics and antimimics may be used to treat drug
20 addiction.

 B) Genes encoding specific enzymes including Catechol-O-methyltransferase (COMT), Synaptagmin III, Beta-adrenergic receptor kinase, Ras-related GTPase (Rab3), Ras-related GTPase beta S-100, aromatic L-amino acid decarboxylase
25 (DOPA decarboxylase) and G-protein beta-1 subunit (rGbeta1);

 These enzymes and their activators and inhibitors may be used to treat drug addiction.

 C) Genes encoding ion channels including K⁺ channel beta subunits (Kv1-type), Na⁺ channel beta 2 subunit (Scn2b),
30 voltage gated K⁺ channel Kv3.4, Shaw-related subfamily member 2 (Kcnc2), K⁺ channel delayed rectifier (RCK2), K⁺ inward rectifier 10 (Kir 4.1), and Ca⁺⁺ channel alpha 1 subunit (Cacna1);

These ion channel proteins and their blockers and activators may be used to treat drug addiction. It should be noted that, for instance, Novartis has an inhibitor of COMT Comtan (Entacapone) used for the treatment of Parkinson.

- D) Genes encoding receptors, which overlap but are not coterminus with the receptors mentioned in A, and which include AMPA receptor GluR1, Kainate receptor KA1, Peripheral benzodiazepine receptor (PKBS), alpha 2-Adrenergic receptor (RG20), NMDA receptor subunit 2, NMDA receptor-like complex glutamate binding protein (GBP), non-process neurexin 1-beta mRNA, GABAA receptor alpha 3 subunit, MAP1A, and NMDA 2D receptor;

These receptors and their agonists and antagonists may be used to treat drug addiction.

- E) Genes encoding receptor-coupled proteins;

These receptor coupling proteins and their activators, inhibitors, agonists and antagonists may be used to treat drug addiction.

- F) Genes encoding transporters exemplified by the vesicular inhibitory amino acid transporter (SVIAAT), Na⁺ dependent high affinity glutamate transporter (GLT-1A), and sodium ATPase isoform, potassium ATPase isoform;

These transporters and their activators and inhibitors may be used to treat drug addiction.

- G) ESTs exemplified by AA799879 and AA956149;

The gene products of these EST 's and ligands for such gene products may be used to treat drug addiction.

- H) Genes encoding growth, survival, functional, structural (gsfs) proteins exemplified by Bcl-x alpha, signal transducer and activation of transcription 3, Retinoblastoma protein, Nsyndecan (syndecan-3 or Neuroglycan), EST 189376,

Synaptotagmin VIII, calcium ion binding protein, and
microtubule-associated protein (MAP1A);

These gsfs proteins and their activators and inhibitors
may be used to treat a drug addiction.

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Preparation of Proteins, Oligopeptides and Peptides of A Through G

The gene expression products of A through G (see Table I) above may be
proteins, shorter oligopeptides or short peptides. All may be generally
characterized as polypeptides. Consequently, that term is used in this section as
10 a synonym for proteins, oligopeptides and peptides. The polypeptides can be
expressed *in vivo* through use of prokaryotic or eukaryotic expression systems.
Many such expressions systems are known in the art and are commercially
available. (Clontech, Palo Alto, CA; Stratagene, La Jolla, CA). Examples of such
systems include, but are not limited to, the T7-expression system in prokaryotes
15 and the baculovirus expression system in eukaryotes. Such expression systems
are well known and have been described. Sambrook and Russell, Molecular
Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold
Spring Harbor, New York, 2001.

Polypeptides can also be synthesized *in vitro*, e.g., by the solid phase
20 peptide synthetic method or by *in vitro* transcription/translation systems. The
synthesis products may be fusion polypeptides, i.e., the polypeptide comprises
the polypeptide variant or derivative according to the invention and another
peptide or polypeptide, e.g., a His, HA or EE tag. Mimics and antimimics may
also be synthesized *in vivo* or *in vitro*. Mimics are generally molecules that
25 mimic the structure of a ligand that is bound by a receptor. Thus, mimics are
generally used to bind and stimulate a receptor. Antimimics are generally
molecules that mimic the structure of a ligand bound by a receptor that decrease
the activity of a receptor upon binding. Methods to synthesize polypeptides are
described, for example, in U.S. Patent Nos. 5,595,887; 5,116,750; 5,168,049 and
30 5,053,133; Olson et al., Peptides, 9, 301, 307 (1988). The solid phase peptide
synthetic method is an established and widely used method, which is described
in the following references: Stewart et al., Solid Phase Peptide Synthesis, W. H.
Freeman Co., San Francisco (1969); Merrifield, J. Am. Chem. Soc., 85 2149
(1963); Meienhofer in "Hormonal Proteins and Peptides," ed.; C.H. Li, Vol. 2

(Academic Press, 1973), pp. 48-267; Bavaay and Merrifield, "The Peptides," eds. E. Gross and F. Meienhofer, Vol. 2 (Academic Press, 1980) pp. 3-285; and Clark-Lewis et al., Meth. Enzymol., 287, 233 (1997). These polypeptides can be further purified by fractionation on immunoaffinity or ion-exchange columns; ethanol precipitation; reverse phase HPLC; chromatography on silica or on an anion-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; or ligand affinity chromatography.

10 Method for Screening

The invention includes a method to determine if a pharmaceutical agent is able to act as an agonist, antagonist, inhibitor, blocker, activator, mimic or antimimic of a gene product or, in the case of a signaling molecule, the associated receptor. In this instance, a pharmaceutical agent may be a peptide, oligopeptide or organic small molecule of any kind. The method can be used to determine if the pharmaceutical agent increases, decreases, activates, blocks, inhibits, mimics or prevents the action of the gene product. The method may be conducted under *in vivo* or *in vitro* conditions.

Potential pharmaceutical agents can be screened *in vivo* for their ability to decrease drug addiction. This may be done by first offered an animal long-term access to an addicting drug such that the animal exhibits an altered mRNA expression profile when compared to animals offered short-term access to the addicting drug and non-exposed control animals. Next, one or more potential pharmaceutical agents can be administered to the experimental animal offered long-term access to the addictive drug. The experimental animal can then be sacrificed and mRNAs can be extracted from the brain of the experimental animal and such that the expression levels in individual genes (such as those described in Table I) may be determined or compared to a control. Methods to determine the expression level of mRNA are known in the art and include, Northern blotting, use of a nucleic acid array or chip, and the like. The expression level of mRNAs extracted from the experimental animal can be compared to those from animals offered short-term access to the addicting drug and to non-exposed control animals. Increased expression in response to the potential pharmaceutical agent of an mRNA that is decreased in an addicted

animal indicates that the potential pharmaceutical agent acts to ameliorate addiction. Also, decreased expression in response to the potential pharmaceutical agent of an mRNA that is increased in an addicted animal indicates that the potential pharmaceutical agent acts to ameliorate addiction.

5 *In vitro* methods may also be used to screen a potential pharmaceutical agent for the ability to ameliorate drug addiction. For example, an *in vitro* method can involve contacting a pharmaceutical agent with a cell that expresses a gene encoding a product included within groups A through H and/or Tables 1 and 2. Altered expression of an mRNA in response to the potential
10 pharmaceutical agent may be determined by extracting mRNA from the contacted cell and comparing expression of a selected mRNA to that in a control cell that was not contacted with the potential pharmaceutical agent.

 The methods of the invention may be used under nearly any conditions wherein a potential pharmaceutical agent can come into contact with a cell. For
15 example, the cells in contact with the potential pharmaceutical agent may be grown on plates, grown in liquid culture, grown in monolayers, or be located in vivo within the body of an organism. Large or small numbers of cells may be used within the methods of the invention. Methods to culture cells are well known in the art and are disclosed herein. Parameters, such as the temperature,
20 time, growth media, pH, and atmosphere used during incubation of the cells with the potential pharmaceutical agent may be adjusted to accommodate specific cell types according to well known procedures.

 The methods of the invention also include the use of detectable labels that can be used to detect binding events, such as those occurring during the
25 binding of a ligand, such as a signaling molecule, by a receptor (such as those disclosed in Table I). In one example, a signaling molecule encoded by an mRNA having expression that is increased or decreased in response to drug addiction may be labeled with a detectable label. A potential pharmaceutical agent can then be added to a mixture containing a cell that expresses a receptor
30 to the labeled signaling molecule and incubated under conditions wherein the receptor can bind to the ligand. The incubation mixture can then be washed and the amount of labeled ligand bound to the cell can be determined through detection of the detectable label. Such methods allow potential pharmaceutical

agents to be screened for their ability to increase or decrease binding of a ligand by a receptor and ameliorate drug addiction.

Numerous detectable labels are known in the art and include, fluorescent proteins, enzymes, antigenic tags, and the like. Such labeled ligands may be expressed within a cell from an exogenous nucleic acid segment. For example, a vector may encode a ligand that is linked to a fluorescent protein and used to express the labeled ligand in a cell. A nucleic acid segment introduced into a cell may encode one or more detectable labels. In addition, a nucleic acid segment introduced into a cell may encode gene products other than detectable labels. Recombinant nucleic acid techniques, cloning vectors, and cellular transformation methods are well known in the art and have been described. Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 3rd edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (2001).

Numerous types of cells may be utilized within the methods of the invention. Such cells can be engineered to allow expression of a desired nucleic acid segment, such as a detectable label. Naturally occurring and immortalized cells may be used within the invention. Genetically modified cells may also be used within the methods of the invention. For example, a cell may be transformed with a nucleic acid construct that directs the expression of a gene product of A through G (as described in Table I) not normally expressed by the cell. Accordingly, genetically modified cells can be constructed to express selected receptors for the potential pharmaceutical agent. Thus, genetically modified cells may be matched with potential pharmaceutical agents and used within the methods of the invention. Such combinations allow one of skill in the art to produce genetically modified cells and gene products that may be used to identify potential pharmaceutical agents.

Use of the in vitro methods of the invention to screen potential pharmaceutical agents may provide any number of results including blockage, activation, inhibition, increasing, decreasing, augmenting and catalyzing gene product function. Use of a single screen will also be effective for identification of potential pharmaceutical agents.

Qualitative and quantitative assays may be conducted. Both will determine whether the interaction sought has occurred. Quantitative assays will

enable identification of an increase, decrease or augmentation of gene product function.

The typical assay will be based upon the function of the gene product involved. For signaling molecules, the appropriate receptor will also be present.

5 This receptor may include its natural enzyme domain to convert the detectable label or may be re-engineered to convert the detectable label. Alternatively, an antibody assay for the bound and/or unbound forms of the signaling molecule may be used. In such an assay, the detection of the detectable label produced by the receptor or through the antibody assay will indicate activity of the candidate.

10 For enzymes, the enzymatic activity may be employed in combination with a detectable label to determine potential pharmaceutical agent interaction. Incorporation of a detectable label into a substrate for the enzyme where the detectable label is released upon enzymatic activity will provide an appropriate *in vitro* assay. The potential pharmaceutical agent activity for activation,
15 inhibition and the like of the enzyme can then be determined by measuring the quantity of detectable label produced.

For ion channels, incorporation into an artificial membrane and determination of the ability of the membrane to pass the appropriate ions may be employed as an appropriate *in vitro* assay. This assay mimics an *in vivo* assay
20 using the degree of ion passage through an appropriate cellular membrane.

Receptors and receptor-coupled proteins may be assayed as described above for signaling molecules. In these instances, the downstream action of an enzymatic domain or triggered enzyme may be employed to appropriate advantage for assaying these gene products according to the invention.

25 Transporter molecules may be assayed for their ability to transport their corresponding substrate molecule which has been modified with a detectable label. An intact cellular membrane or artificial membrane may be employed as the functional system in which the transporter molecule operates. Assay of the detectable label delivered, or not delivered across the membrane by the
30 transporter molecule will identify potential pharmaceutical agents interacting with these molecules.

Many methods may be used to detect the detectable label. Chemiluminescence may be used to detect the detectable label. Briefly, the detectable label can be contacted with a substrate that is acted upon by the

detectable label to produce a signal that may be detected with a luminometer. For example, the following detectable labels and their substrates are provided as examples that may be used for chemiluminescent detection of cellular invasion: alkaline phosphatase with AMPPD; β -galactosidase with AMPGD; horseradish peroxidase with luminol + perborate + 4-iodophenol; and xanthine oxidase with luminol + Fe EDTA (Harlow et al., Antibodies: A Laboratory Manual, page 319 (Cold Spring Harbor Pub. 1988)). Bioluminescence may be used in an analogous manner as chemiluminescence to detect a detectable label. Fluorescence may be used to detect a fluorescent protein that is produced, transported, converted or expressed as a detectable label. For example, green fluorescent protein may be the result of any of the foregoing *in vivo* or *in vitro* assays and may be detected with a fluorimeter, a fluorescent plate reader, or a fluorescent microscope. Ultraviolet or visible light may be used to detect the presence of a detectable label produced in an assay according to the invention. Such detection methods are known in the art and are disclosed herein.

Agonists, Antagonists, Activators, Blockers, Inhibitors, Ligands, Anti-ligands, Anti-signaling molecules, Mimics, and Anti-mimics (see 1-9 above) of Proteins A through H

Secretases (sheddases) can be useful as therapeutic targets in cocaine addiction. Several proteins have been identified as members of a diverse range of membrane proteins that also occur as soluble forms derived from the membrane form by proteolysis. Protease cleavage regulates the activity of these proteins. Inhibition of protease cleavage of the ectodomains of these proteins could interfere with the biological process induced by the escalation of cocaine addiction. Proteolytic cleavage of the ectodomains of these membrane proteins is carried out by a group of enzymes referred to collectively as 'secretases' or 'sheddases'. The majority of secretases are matrix metalloproteases (MMP). These shed membrane proteins identified as being induced during the escalation of cocaine addiction include, but are not limited to, syndecan 3, fractalkine, and TNF receptor (p60), which ligand TNF-alpha is also regulated by proteolytic cleavage of its ectodomain. Additionally, PDGF-A was found to be increased and the PDGF receptor ectodomain is also released by protease cleavage. The notion that dysregulation of the secretase system could be induced by the

escalation of cocaine addiction is also supported by the observation that tissue inhibitor metalloproteinase 3 (TIMP-3) was found to be increased by escalation of cocaine intake. TIMP-3 has been shown to inhibit syndecan 3 cleavage and, like syndecan, it is increased by food deprivation (Reizes O., 2003). TIMP-3 preferentially inhibits MMP-1, -3, -7, -13 and the TNF-alpha-converting enzyme (TACE) (Stamenkovic, 2003), although inhibitory activities of different TIMPs towards different MMPs are not particularly selective. Notably, PDGF-A has been shown to upregulated MMPs in some tissues (Robbins 1999). Many proteins released by ectodomain cleavage have been previously disclosed to be involved in pathophysiological processes such as neurodegeneration, apoptosis, oncogenesis and inflammation, and therefore secretases have received great attention as possible therapeutic targets. In addition, another tissue protease system, the tissue plasminogen activator (t-PA) was found to be induced. T-PA has been implicated in synaptic plasticity (discussed in Nicholas, 2003) and potentiates NMDA-receptor function (Nicole, 2001).

TNF receptor (p60): The observed decrease in TNF receptor (p60) may reflect induction of TNF-alpha. Shedding of membrane-bound pro-TNF-alpha is thought to be largely due to TNF-alpha-converting enzyme (TACE), therefore TACE inhibitors could be beneficial. Large collections of MMP inhibitors, including TACE inhibitors are being developed by several companies (reviewed in Hooper 1997). (For example, see <http://www.uspto.gov/> for patent and patent publications that are assigned to Pfizer (Letavic et al. 2003), Wyeth Research (Levin et al. 2001a, 2001b, 2002 and 2003; Zask et al. 2003; Nelson et al. 2003; Chen 2002), Glaxo Wellcome (Conway et al. 2001), Immunex Corporation (Mullberg, 1995) and Bristol-Myers (Duan et al 2002), such patents and patent publications are hereby incorporated by referenced).

Fractalkine: Fractalkine acts as a neuron- or endothelial- derived intercellular signaling molecule to attract proinflammatory cells after excitotoxic injury, such events are amplified by fractalkine cleavage, which is promoted by TNF-alpha and other cytokines. Blocking fractalkine cleavage with the secretase inhibitor Batimastat (AKA BB94, Glaxo-SmithKline) inhibits these events (Chapman , 2000).

PDGF-A and the PDGF-alpha receptor (PDGFR-alpha) are present in various neuronal populations in the adult CNS. PDGF receptor inhibitors have

been established as antitumor drugs, including several tyrphostin compounds like AG1295, AG-1296 (Levitzki A 1999, Lipson 1998).

Syndecan 3: As discussed above, the activity of syndecan can be modulated by secretases. During food deprivation, TIMP-3 is induced, resulting in inhibition of a sheddase or matrix metalloprotease, leading to an increase in cell surface expression of syndecan-3. Similarly, it was observed that both Syndecan 3 and TIMP-3 were induced in cocaine escalating rats (Reizes, 2003). Exogenous matrix metalloprotease inhibitor or increased TIMP-3 expression results in increased syndecan-3 expression and increased food intake (Reizes, 2003). Syndecan 3 has been shown to increase the action of the orexigenic peptide AGRP which acts as an endogenous competitive antagonist of alpha-melanocyte-stimulating hormone (alpha-MSH) at the melanocortin-3 and -4 receptors. This analogy between the systems controlling food intake and drug abuse suggests that drugs being developed to treat obesity by acting on orexigenic (Ghrelin, NPB/NPW, AGRP, NPY, MCH, Orexyn A/B, galanin/GALP, Beacon, beta-endorphin, dynorphin, GHRF) and anorexigenic (alpha-MSH, CART, PYY3-36, NPB, CRF, urocortin II, III, GLP-I, oxytocin, neurotensin, CCK, GRP, bombinakinin-GAP, neuromedin, POMC, ADM, somatostatin, TRH, CGRP) peptide systems could also be beneficial in drug abuse. Prior to Applicants' invention, AGRP, the peptide most likely to be directly regulated by syndecan has not previously been associated with drugs of abuse, including cocaine. However, Lindblom et al (May 2002) suggest that the AA strain of alcohol preferring rats have a high ratio of POMC/AGRP expression, and that this observation is accompanied by differences in MC3 receptor levels. Also, the non-selective MC-receptor agonist MTII caused a reduction in ethanol intake and ethanol preference in AA rats (Ploj K 2002 Oct). Earlier work had implicated the melanocortins in opiate addiction (Alvaro 1997) and recently in the effects of cocaine (Alvaro 2003), which appear to be opposite to those of opiates (morphine down-regulates the expression of MC4-R in striatum and periaqueductal gray while cocaine up-regulates MC4-R mRNA expression in the striatum and hippocampus (Alvaro 2003)). However, AGRP had not been previously associated with cocaine addiction and nor have there been any studies on the regulation of these systems in the hypothalamus where changes in syndecan regulation were demonstrated herein.

Tissue plasminogen activator (t-PA): t-PA was increased in the lateral hypothalamus of cocaine escalating rats, while plasminogen activator inhibitor 2 (PAI-2) was slightly decreased. Plasminogen activators convert plasminogen to the active protease plasmin and have been previously implicated in brain plasticity and in toxicity inflicted in hippocampal pyramidal neurons by kainate (Sharon 2002) and hypoxia (Hosomi 2001). Additionally, t-PA potentiates signaling by glutamatergic receptors by cleaving the NR1 subunit of the NMDA receptor resulting in a 37% increase in NMDA-receptor function. These results were confirmed in vivo by the intrastriatal injection of recombinant-PA, which potentiated the excitotoxic lesions induced by NMDA (Nicole 2001). A role for t-PA in neural plasticity is supported by observations that t-PA overexpression improves water maze performance, additionally long-term potentiation (LTP) induction in hippocampal slices is associated with an increase in tPA expression, and inhibitors of tPA activity impair late-phase LTP in hippocampal slices (discussed in Nicholas, 2003). A synthetic tPA/plasmin inhibitor is called tPA-stop (America Diagnostica Inc. #544).

IGF: Both pharmacological inhibitor and gene therapy approaches are being developed to inhibit the IGF system as antitumor strategies. A pharmacological example is Tyrphostin AG 1024 (Parrizas et al 1997) and an example of gene therapy strategy is disclose in Johnson et al. (1994).

Stat 3: The JAK family-specific inhibitor, tyrphostin AG490, markedly inhibits Stat3 activation (Toyonaga, 2003; Zhang 2000).

IL-3: Mice transgenic for IL-3 under the control of the GFP promoter develop progressive motor disease at approximately 5 months. Lesions identified after disease onset showed activation of microglia, astroglial proliferation with phagocytosis of lipids, and immigration of macrophages and mast cells into neural parenchyma. Therefore overexpression of IL-3 in cocaine escalation could contribute to microglia activation and promotion of inflammation. Agents that inhibit microglia proliferation include, but are not limited to, the aforementioned inhibitors of the shedding of fractalkine and could be beneficial by countering the action of IL-3. The JAK family-specific inhibitor, tyrphostin AG490 that inhibits Stat3 activation (Toyonaga, 2003; Zhang 2000) also blocks most effects of IL-3 (Si and Collins 2002).

Kv3.4 blockers: tetraethylammonium (TEA), 4 aminopyridine (4AP), BDS.

Kir4.1 blockers: barium.

K⁺ channel beta subunit inhibitor: calphostin C.

- 5 Periferal Benzodiazepine receptor (PKBS): PKBS has been known to have many functions such as a role in cell proliferation, cell differentiation, steroidogenesis, calcium flow, cellular respiration, cellular immunity, malignancy, and apoptosis. Its expression in the brain mostly reflects astrocytes and microglia activation (Versijpt , 2003). Ligands include, in order of affinity:
- 10 PK11195 = Ro5-4864 > FGIN-1-27 > triazolam = diazepam > beta-pro-pyl-beta-carboline-3-carboxylate = clonazepam > lorazepam = flurazepam >> chlordiazepoxide = clorazepate. Treatment with peripheral (Ro5-4864) and mixed (diazepam), but not central (clonazepam), benzodiazepine receptor ligands blocked certain aspects of microglia activation (Lokensgard 1998, 2001).
- 15 PK11195 is used for visualization of neuroinflammation in vivo (Cagnin A, 2002).

GluR1: AMPA receptor inhibitors NBQX, CNQX, LY300168
GYKI53655.

Kainate receptor antagonists: CNQX at high dose, 3-CBW.

- 20 GABAA alpha3 subunit: the GABA agonist Gabapentin.

Pharmaceutical Compositions

- According to the invention, the gene products and the related agonists, antagonists, activators, blockers, inhibitors, ligands, mimics, antimimics of A
- 25 through H above may be chemically configured as proteins, oligopeptides and small organic molecules. Together, these compounds will be discussed in this section as proteins and related molecules. The proteins and related molecules of the invention may be formulated into a variety of acceptable compositions. Such pharmaceutical compositions can be administered to a mammalian host,
- 30 such as a human patient, in a variety of forms adapted to the chosen route of administration, *i.e.*, orally or parenterally, by intravenous, intramuscular, topical or subcutaneous routes.

In cases where the proteins and related molecules are sufficiently basic or acidic to form stable nontoxic acid or base salts, administration of such

proteins and related molecules, as salts may be appropriate. Examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids that form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, 5 ascorbate, α -ketoglutarate, and α -glycerophosphate. Suitable inorganic salts may also be formed, including hydrochloride, sulfate, nitrate, bicarbonate, and carbonate salts.

Pharmaceutically acceptable salts are obtained using standard procedures well known in the art, for example by reacting a sufficiently basic 10 compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example calcium) salts of carboxylic acids also are made.

Thus, the present proteins and related molecules, may be systemically 15 administered, *e.g.*, orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the proteins and related molecules, may be 20 combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the 25 weight of a given unit dosage form. The amount of oxidants and oxygen scavengers in such therapeutically useful compositions is such that an effective dosage level will be obtained.

The tablets, troches, pills, capsules, and the like may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; 30 excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in

addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the active compound, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and devices.

The proteins and related molecules may also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the proteins and related molecules may be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the proteins and related molecules that are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be

brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the proteins and related molecules in the required amount in the appropriate solvent with
5 various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the oxidants and oxygen scavengers plus any additional desired ingredient present in the
10 previously sterile-filtered solutions.

For topical administration, the proteins and related molecules may be applied in pure form, i.e., when they are liquids. However, it will generally be desirable to administer them to the skin as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or
15 a liquid.

Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina and the like. Useful liquid carriers include water, alcohols or glycols or water-alcohol/glycol blends, in which the present compounds can be dissolved or dispersed at effective levels, optionally
20 with the aid of non-toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers.

25 Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user.

Useful dosages of the proteins and related molecules of the present
30 invention can be determined by comparing their *in vitro* activity, and *in vivo* activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Pat. No. 4,938,949.

Generally, the concentration of the proteins and related molecules of the present invention in a liquid composition, such as a lotion, will be from about 0.1-25 wt-%, preferably from about 0.5-10 wt-%. The concentration in a semi-solid or solid composition such as a gel or a powder will be about 0.1-5 wt-%,
5 preferably about 0.5-2.5 wt-%.

The amount of the proteins and related molecules or an active salt or derivative thereof, required for use in treatment will vary not only with the particular salt selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be
10 ultimately at the discretion of the attendant physician or clinician.

In general, however, a suitable dose will be in the range of from about 0.5 to about 100 mg/kg, e.g., from about 10 to about 75 mg/kg of body weight per day, such as 3 to about 50 mg per kilogram body weight of the recipient per day, preferably in the range of 6 to 90 mg/kg/day, most preferably in the range
15 of 15 to 60 mg/kg/day.

The proteins and related molecules are conveniently administered in unit dosage form; for example, containing 5 to 1000 mg, conveniently 10 to 750 mg, most conveniently, 50 to 500 mg of active ingredient per unit dosage form.

Ideally, the proteins and related molecules should be administered to
20 achieve peak plasma concentrations of the proteins and related molecules of from about 0.005 to about 75 μ M, preferably, about 0.01 to 50 μ M, most preferably, about 0.1 to about 30 μ M. This may be achieved, for example, by the intravenous injection of a 0.05 to 5% solution of the proteins and related molecules, optionally in saline, or orally administered as a bolus containing
25 about 1-100 mg of the proteins and related molecules. Desirable blood levels may be maintained by continuous infusion to provide about 0.01-5.0 mg/kg/hr or by intermittent infusions containing about 0.4-15 mg/kg of the proteins and related molecules.

The desired dose may conveniently be presented in a single dose or as
30 divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, e.g., into a number of discrete loosely spaced administrations; such as multiple inhalations from an insufflator or by application of a plurality of drops into the eye.

The therapeutic compositions of this invention, proteins and related molecules that include both engineered proteins and related molecules and other molecules containing additional reductive centers as described herein for promoting proteins and related molecules activity, are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered and timing depends on the subject to be treated, capacity of the subject's system to utilize the active ingredient, and degree of therapeutic effect desired. Precise amounts of active ingredient required to be administered depend on the judgement of the practitioner and are peculiar to each individual. However, suitable dosage ranges for various types of applications depend on the route of administration. Suitable regimes for administration are also variable, but are typified by an initial administration followed by repeated doses at intervals to result in the desired outcome of the therapeutic treatment.

Therapeutic compositions of the present invention contain a pharmaceutically acceptable carrier together with the proteins and related molecules. In a preferred embodiment, the therapeutic composition is not immunogenic when administered to a mammal or human patient for therapeutic purposes.

The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art and need not be limited based on formulation. Typically such compositions are prepared as injectables either as liquid solutions or suspensions, however, solid forms suitable for solution, or suspensions, in liquid prior to use can also be prepared.

The preparation can also be emulsified.

The active ingredient can be mixed with excipients that are pharmaceutically acceptable and compatible with the active ingredient and in amounts suitable for use in the therapeutic methods described herein. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like which enhance the effectiveness of the active ingredient.

The therapeutic compositions of the present invention can include pharmaceutically acceptable salts of the components therein. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide) that are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, tartaric, mandelic and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine and the like.

Pharmaceutically acceptable carriers are well known in the art. Exemplary of liquid carriers are sterile aqueous solutions that contain no materials in addition to the active ingredients and water, or contain a buffer such as sodium phosphate at physiological pH value, physiological saline or both, such as phosphate-buffered saline. Still further, aqueous carriers can contain more than one buffer salt, as well as salts such as sodium and potassium chlorides, dextrose, polyethylene glycol and other solutes.

Liquid compositions can also contain liquid phases in addition to and to the exclusion of water. Exemplary of such additional liquid phases are glycerin, vegetable oils such as cottonseed oil, and water-oil emulsions.

The invention is further described in detail by reference to the non-limiting examples that follow. While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which is described and claimed.

Exemplary Protocol

In rats allowed to self-administer cocaine, the duration of access dramatically influenced cocaine intake. Within 18 days, the first hour of cocaine intake in LgA rats rose to a level almost two times greater than that observed in ShA rats, which, as expected, remained stable over time (Fig. 1). Total intake in LgA rats also increased over the same period of time from an initial average of 48 to 126 cocaine injections. Forty-eight hours after the last self-administration session, all animals were sacrificed to obtain tissue samples from 6 reward-

related brain regions: ventral tegmental area (VTA), lateral hypothalamus (LH), amygdala (AMG), nucleus accumbens (ACC), septum (SEP) and prefrontal cortex (PFC). Gene expression profiling was then performed for each dissected brain region using the Affymetrix Rat Neurobiology Array. This array consists
 5 of over 1300 probe sets representing all known neurotransmitter receptors, transporters, synthetic and metabolic enzymes, signal transduction proteins, as well as other brain-specific transcripts. Relative variations from control levels in ShA and LgA probe sets are plotted together in Fig. 2. Regression analysis showed a positive correlation gene expression changes between cocaine-exposed
 10 groups (all r values were above 0.43, $p < 0.01$); this correlation was the lowest in the nucleus accumbens ($r = 0.20$, $p < 0.01$). Thus, regardless of the brain region considered, the majority of genes whose expression levels are affected after exposure to cocaine self-administration were not differentially affected by the pattern of cocaine intake (stable/moderate in ShA rats vs. escalating/excessive in
 15 LgA rats).

As shown in Tables 1 and 2, ES genes can be classed in four functional categories: 1) genes coding for proteins involved in the regulation of neuronal growth, survival and functional and structural plasticity; 2) genes coding for proteins involved in the regulation of membrane potential such as ion pumps and
 20 channels; and 3) neurotransmitter receptors, synthetic and metabolic enzymes and transducers; and 4) genes involved in the neurotransmitter release machinery. For convenience, the tabular chart presenting this information has been divided into Tables 1 and 2. The graphs of Table 2 correlate with the charted information of Table 1 as indicated by the gene listings. Consequently,
 25 the graphs of Table 2 align with the rows of Table 1 according to the gene names.

Table 3 presents the results of hybridization of the lateral hypothalamus with Affymetrix chip: RAE-23OA expression array (the last 3 were obtained with the dChip analysis software that is logarithmic and therefore significance is
 30 obtained with lower fold changes). The columns are: probe set (Affymetrix id of probes on the chip); accession number (general identifier for the gene sequence from which the probe is derived); FC C/A (fold change between condition C (cocaine escalating rats) and A (control)); FC C/B (fold change between condition C (cocaine escalating rats) and B (cocaine NON escalating rats); Gene

(name of the gene); and Software used to generate the fold change value (MAS 5.0 or dChip 1.3).

Table 4 discloses a large number of candidate genes that appear to be associated with the development of the escalation of cocaine intake/addiction.

5 The data presented in Table 4 is the product of repeated analysis with various algorithms. The columns are: probe set (Affymetrix id of probes on the chip); accession number (general identifier for the gene sequence from which the probe is derived); FC C/A (fold change between condition C (cocaine escalating rats) and A (control)); FC C/B (fold change between condition C (cocaine escalating rats) and B (cocaine NON escalating rats); and title (name of the gene).

The lateral hypothalamus was the brain structure that revealed the greatest changes in gene expression. Several genes involved in structural plasticity changed with cocaine escalation in this area. Examples of such genes are the alpha2 and beta2 isoforms of Na⁺, K⁺-ATPase isoforms, which have
15 been shown to be induced in Schwann cells during peripheral nerve regeneration (Kawai et al., 1997); the proteoglycan N-syndecan (syndecan-3 or neuroglycan), which is transiently expressed on growing axons during development and binds heparin-binding growth factors with neurite-promoting activity (Bandtlow and Zimmermann, 2000); Neuroligin 3, a member of a family of synaptically
20 associated adhesion molecules, which has been implicated in synaptogenesis (Cantalupo and Cline, 2000), was also found to be induced in the LH. Increased transcription of the trophic factor PDGF, its transducer STAT3, and the anti-apoptotic factor Bcl-xalpha - whose transcription is regulated by PDGF and STAT3 (Huang et al., 2000; Stephanou et al., 2000) – was also seen in the LH of
25 LgA rats. This coordinate pattern of gene expression changes indicates a response to a pro-apoptotic insult in hypothalamic cells of animals that have developed escalated levels of drug intake. The transcript for the chemokine fractalkine was also upregulated in the LH of escalating rats. Fractalkine is a chemokine predominantly expressed in the brain, which is believed to be part of
30 a mechanism response to excitotoxic neuronal injuries (Chapman et al., 2000). Both fractalkine and PDGF reduce glutamate neurotransmission and their activation could be a response to chronic activation of glutamate-mediated excitatory neurotransmission (Chapman et al., 2000; Sims et al., 2000).

Changes in the expression of selected glutamate receptors were also observed. In particular, in the lateral hypothalamus, expression of the AMPA receptor subunit 1 (GluR1) was decreased and expression of N-methyl-D-Aspartate receptor subunits 2D (NR2D) was increased in rats that have
5 developed escalated levels of cocaine intake. Expression of GluR1 has been found to be increased in the VTA following repeated administration of morphine and cocaine (Carlezon et al., 1997) and viral mediated overexpression of this receptor in the VTA induces sensitization to morphine (Carlezon et al., 2001). Interestingly, however, intracranial self stimulation in the LH has been shown to
10 decrease GluR1 expression in the VTA (Carlezon et al., 2001). GluR1 expression was not significantly increased in the VTA in both LgA and ShA rats (not shown). GluR2 was significantly decreased in both LgA and ShA rats in the LH (not shown). The messenger for kainate-type glutamate receptor 1 (KA1) was also decreased in escalating rats. The down-regulation of GluR1 is also a
15 response to chronic activation of glutamate-mediated neurotransmission.

The NR2D subunit is predominantly expressed during development and confers slow channel kinetics to the NMDA receptors (Cull-Candy et al., 2001; Monyer et al., 1994; Vicini and Rumbaugh, 2000). The slow deactivation of the embryonic subunits is believed to lower the temporal threshold for coincidence
20 detection favoring synaptic strengthening during development (Cull-Candy et al., 2001; Monyer et al., 1994; Vicini and Rumbaugh, 2000). Extrasynaptically located NR2D receptors have been demonstrated (Misra et al., 2000). Such extrasynaptic NR2D receptors are thought to mediate glutamate trophic actions rather than contributing to neural transmission (Misra et al., 2000; Vicini and
25 Rumbaugh, 2000). Thus, the increased expression of the embryonic NR2D subunit in the lateral hypothalamus of cocaine escalating rats could be a hallmark of plastic structural rearrangements.

Alterations in the expression of different K⁺ channels suggest changes in cellular excitability in the LH. Particularly in cocaine-escalating rats, the
30 expressions of a delayed rectifier, an A-type potassium channel (Kv3.4), and an inward rectifier were increased. Delayed rectifiers reduce cellular excitability by increasing action potential threshold, while both delayed rectifiers and A-type channels act by reducing the duration of action potentials resulting in increased frequency of firing (Coetzee et al., 1999). This firing characteristic is usually

associated with inhibitory interneurons (Coetzee et al., 1999). The Kv3.4 channel is sparsely expressed, but has been shown to be expressed in the subthalamic nucleus, whose neurons have characteristics of both projection neurons and interneurons and contribute to the regulation of midbrain
5 dopaminergic neurons (Rudy et al., 1999). Inward rectifiers have been involved in opioid inhibition of locus coeruleus neurons (Nestler and Aghajanian, 1997). The Kir4.1 inward rectifier channel has also been implicated in neuronal development and differentiation (Neusch et al., 2001). Increased expression of the vesicular inhibitory amino acid transporter in the LH of cocaine-escalating
10 rats was also observed. The vesicular inhibitory amino acid transporter is a marker of inhibitory synapses (Dumoulin et al., 1999) and its increased expression could suggest increased synaptic terminals from inhibitory interneurons.

The G-protein beta subunit rGbeta1, was found to be downregulated in
15 the LH of escalating rats. Interestingly, this G-protein beta subunit is upregulated by cocaine or amphetamine in the shell region of the nucleus accumbens and it is required for behavioral sensitization induced by repeated administration of psychostimulants (Wang et al., 1997).

The expression levels of only a small fraction of genes changed
20 specifically in association with drug intake escalation (ES genes). The most dramatic changes were observed in the lateral hypothalamus. This observation points to a previously under-appreciated importance of this hypothalamic area in the development of drug addiction. Most of the ES genes identified encode for proteins normally involved in key neurodevelopmental processes, including
25 neurite extension and synaptogenesis differentiation and apoptosis. Genes involved in such processes are increasingly recognized as mediators of plasticity and regeneration in the adult brain. A second broad category of genes that was found to be selectively regulated in cocaine escalating animals are genes involved in the regulation of glutamate neurotransmission and neuronal
30 excitability. The concurrent changes in these two categories of genes during cocaine intake escalation indicates that they are an adaptation to a common perturbation. The present observations show that escalation of cocaine intake is associated with changes in brain structure and function does not depend on a single gene, but on an intricate interplay of multiple genes involved in plastic

rearrangement of neural connections and transmission and that neuroadaptive changes in response to chronic activation of glutamate-mediated excitatory neurotransmission could be present in the lateral hypothalamus of rats with escalated cocaine intake.

5 **Behavioral procedure.** Twenty-eight male Wistar rats (280-340 g) were prepared with a chronic intravenous catheter and 5 days later were food-restricted and trained for 7 days to press a lever to obtain food pellets. Two days after food-training, 20 rats were tested for cocaine self-administration during two consecutive phases: a *screening phase* (1 day) and an *escalation phase* (18 days).
10 The remaining 8 rats were exposed to the same experimental manipulations as the other rats, except that they were not exposed to cocaine. During the *screening phase*, the 20 rats tested for self-administration were allowed to self-administer cocaine during only one hour on a fixed-ratio 1 schedule (250 µg/injection in a volume of 0.1 ml delivered in 4 sec) after which two balanced
15 groups with the same mean weight and mean cocaine intake were formed. During the *escalation phase*, one group had access to cocaine self-administration for only 1 hour per day (Short-Access or ShA rats) and the other group for 6 hours per day (Long-Access or LgA rats). Four out of the 20 rats allowed to self-administer cocaine were discarded from the study either because of a failure to
20 reach the criterion for acquisition of cocaine self-administration (n=3) (i.e., at least 8 injections per hour) or because of inconsistent within-session intake for several days (n=1), leaving 8 rats per group.

Brain dissection. Drug-naïve, ShA and LgA rats (8 per group) were sacrificed in random order following anesthesia by CO₂ narcosis and perfused
25 with 10% RNA Later (Ambion) in phosphate buffered solution. To reduce variation between animals as much as possible, brains were carefully sliced using a wire brain slicer (Research Instruments & MFG, Corvallis OR). Brain slices were then dissected with the assistance of a brain atlas. Standardized needle punching was performed to remove the nucleus accumbens (ACC), the
30 lateral hypothalamus area (LH), the septum (SEP) and the ventral tegmental area (VTA). The punching needle (14 gauge) was constructed from a modified spinal tap needle and equipped with a plunger. The medial prefrontal cortex (PFC) and the amygdaloid complex (AMG) were dissected free-handedly using established anatomical landmarks. Due to the small size of certain brain regions, tissue

samples from different animals had to be pooled. Pools from 2, 4, or 8 animals were made for AMG and MPF, ACC and LH, and SEP and VTA respectively.

RNA and Probe preparation. Total RNA of regions of interest were prepared using the Qiagen RNeasy miniprep kit according to manufacturer's protocol. Quality of RNA was assessed spectrophotometrically and by agarose gel electrophoresis. Between 1 and 5 micrograms of total RNA were used to prepare double-stranded cDNA (1st & 2nd strand cDNA synthesis components from GibcoBRL). Biotinylated cRNA was transcribed from that cDNA using the BioArray High Yield RNA Transcript Labeling kit (Enzo), purified on RNeasy spin columns (Qiagen), and then fragmented.

Hybridization. Hybridization cocktails were boiled at 99°C, loaded on the Affymetrix Neurobiology RNU34 chips, and hybridized at 45°C for 16 hours. Washes were performed on the Affymetrix Fluidics Station using manufacturer recommended wash solutions and stained with a streptavidin phycoerythrin conjugate to allow for fluorescent detection. After staining, chips were scanned with the Affymetrix Chip Reader at 3µm resolution. For the AMG and PFC, hybridizations were run in quadruplicate (4 independent pools hybridized once each). For the ACC and LH we carried out duplicate hybridizations of 2 pools each (2 independent pools hybridized twice each). For the VTA and SEP, we carried out 3 replicate hybridizations of individual pools (1 pool hybridized 3 times).

Data analysis. Gene expression changes associated with escalated cocaine intake (ES genes) were investigated. ES genes were defined as genes whose expression levels in LgA rats was significantly different ($p < 0.05$) both from control rats and ShA rats. Genes with expression levels different from control levels in both ShA and LgA, but not different between ShA and LgA rats were defined as being associated with cocaine self-administration (SA genes) but not with escalation. Quadruplicate or triplicate results were averaged in each group. Probe sets with mean expression levels below 20 in all three groups were not considered for subsequent analyses and negative expression values were turned to 0. Following previous recommendations (Lockhart and Barlow, 2001), only probe sets displaying significant ($p < 0.05$) changes of 1.8-folds or greater were considered biologically significant. However, probe sets with changes between 1.4 and 1.8 folds were also included if highly significant ($p < 0.01$).

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All publications, patents and patent applications are incorporated herein by reference. While in the foregoing specification this invention has been described in relation to certain preferred embodiments thereof, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details described herein may be varied considerably without departing from the basic principles of the invention.

10

Table 1

Results: Micro Array Data Analysis

Growth/Structure/Plasticity

Gene	UniGene Annotation	Accession	Brain Region	Direction	FC Level	Gene Type	Fisher's post-hoc		Scheffe's post-hoc		Fold Changes	
							Cid vs ShA	Cid vs LoA	Cid vs ShA	ShA vs LoA	ShA vs Cid	ShA vs Cid
Tumor necrosis factor receptor chain (p60)		M63122	ACC	Down	---	Growth/Structure/Plasticity	p<0.05	p<0.01	NS	NS	0.69	0.38
Insulin-like growth factor II	igf2 insulin-like growth factor II (somatomedin A)	X17012	AMG	Down	---	Growth/Structure/Plasticity	p<0.01	p<0.01	p<0.05	NS	0.61	0.59
Fractalkine Chemokine CX3C motif ligand 1	Rattus norvegicus chemokine CX3C mRNA, complete cds (soluble secreted form) cDNA clone RBRGJ76; Cx3cl1 Chemokine (C-X2-C motif) ligand 1 (SYDL RAT Fractalkine precursor (CX3CL1) (Neurotactin) (CX3C membrane-anchored chemokine) (Small inducible molecule)	AF030358/A227647	LH	Up	++	Growth/Structure/Plasticity	p<0.01	p<0.01	p<0.01	p<0.01	1.49	1.91
Platelet-derived growth factor A chain (PDGFA)	Podia Platelet-derived growth factor A chain	D10106	LH	Up	+	Growth/Structure/Plasticity	p<0.01	p<0.01	p<0.01	p<0.05	1.17	1.59
Bcl-2 alpha (Bcl-2 family)		U72350	LH	Up	+++	Growth/Structure/Plasticity	NS	p<0.01	NS	p<0.01	1.25	2.13
Signal transducer and activator of transcription 3 (STAT 3)		X91810	LH	Up	+++	Growth/Structure/Plasticity	p=0.078	p<0.01	NS	p<0.01	1.76	3.4
N-eynoscan (Neuroglycan)	Sdc3 Syndecan	X63143	LH	Up	+++	Growth/Structure/Plasticity	p<0.01	p<0.01	p<0.01	p<0.01	4.94	8.61
Microtubule-associated protein (MAP1A)		M45196	LH	Up	+++	Growth/Structure/Plasticity	p<0.01	p<0.01	p<0.01	p<0.01	1.88	2.19
Regulated GTPase (RAB5)		X08189	LH	Up	++	Growth/Structure/Plasticity	p<0.01	p<0.01	p<0.01	p<0.01	1.35	2.02
Insulin-like growth factor II (somatomedin A)	igf2 insulin-like growth factor II (somatomedin A)	E06910	SEP	Down	+ to -	Growth/Structure/Plasticity	NS	p<0.01	NS	p<0.05	0.72	-0.42
Microtubule-associated protein (MAP1B)	Map1b Microtubule-associated protein 1b	U52950	SEP	Down	---	Growth/Structure/Plasticity	NS	p<0.01	NS	p=0.093	0.89	0.48
Neurofilin 3		U41663	SEP	Down		Growth/Structure/Plasticity	p=0.072	p<0.05	NS	p=0.062	Need FC	
Interleukin-3 beta		U81492	SEP	Up	++	Growth/Structure/Plasticity	NS	p<0.05	NS	p<0.05	1.01	1.84
Retinoblastoma protein (pRb)		D28233	SEP	Up	+	Growth/Structure/Plasticity	p<0.05	p<0.01	p<0.05	p<0.09	1.2	1.39
beta 5-iron Ca++ binding protein		A146214	VTA	Up	+++	Growth/Structure/Plasticity	p<0.05	p<0.01	p<0.078	p<0.01	1.34	2.39

Ion pumps and channels

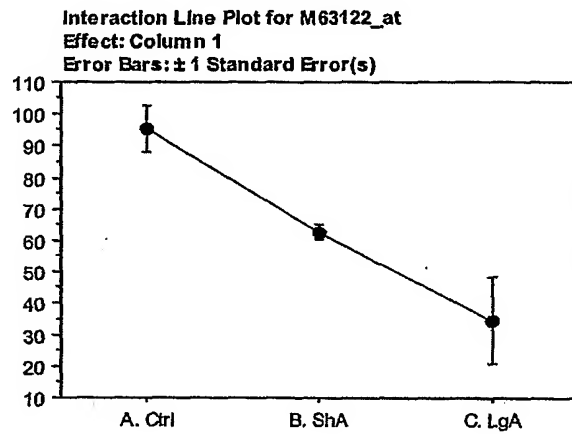
Gene	UniGene Annotation	Accession	Brain Region	Direction	FC Level	Gene Type	Fisher's post-hoc		Scheffe's post-hoc		Fold Changes	
							Cid vs ShA	Cid vs LoA	Cid vs ShA	ShA vs LoA	ShA vs Cid	ShA vs Cid
K+ channel beta subunit (Kv1.2)		X70662	ACC	Down	++	Ion pumps and channels	NS	p<0.01	NS	p<0.01	0.8	0.51
Na+ channel beta 2 subunit (Scn2b)	Scn2b Sodium channel beta 2	U37147	AMG	Up	+++	Ion pumps and channels	NS	p<0.05	NS	p<0.05	1.25	2
Na+/K+-transporting ATPase alpha 2 subunit (Atp1a2)	Atp1a2 ATPase, Na+/K+-transporting, alpha 2	M28648	LH	Up	+++	Ion pumps and channels	p<0.01	p<0.01	p<0.01	p=0.094	2.45	3.11

Table 1

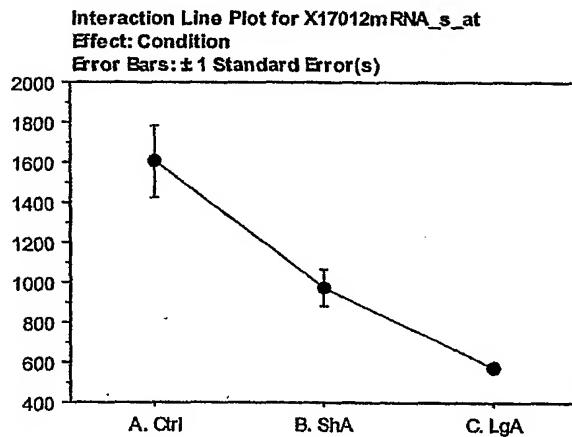
Gene	UniGene Annotation	Accession	Brain Region	Direction	FC Level	Gene Type	Fisher's post-hoc		Scheffe's post-hoc		Fold Changes	
							Sh vs Loh	Sh vs Loh	Sh vs Loh	Sh vs Loh	Sh vs Loh	Sh vs Loh
Voltage-gated K ⁺ channel subunit 2 (Kv2.2)	Kv2.2 Potassium voltage-gated channel, Shaker-related subfamily, member 2	X62841	LH	Up	++	Ion pumps and channels	NS	p<0.01	p<0.01	NS	p<0.01	1.35
K ⁺ channel, delayed rectifier (Kv2.2)	Kv2.2 Potassium voltage-gated channel, Shaker-related subfamily, member 2	X17421/X12518	LH	Up	+++	Ion pumps and channels	NS	p<0.01	p<0.01	NS	p<0.01	1.14
Na ⁺ /K ⁺ -ATPase beta 2 subunit	Na ⁺ /K ⁺ -ATPase beta 2 subunit	D90048/D04529	LH	Up	+++	Ion pumps and channels	p<0.05	p<0.01	p<0.01	NS	p<0.01	1.41
K ⁺ inward rectifier 10 (Kir4.1)	K ⁺ inward rectifier 10 subunit	X5505	LH	Up	+	Ion pumps and channels	p<0.01	p<0.01	p<0.01	p<0.05	p<0.01	0.74
Ca ²⁺ channel alpha 1 subunit (Cacophony)	Ca ²⁺ channel alpha 1 subunit (Cacophony)	U14005	VTA	Up	+++	Ion pumps and channels	NS	p<0.01	p<0.01	NS	p<0.01	1.13
Neurotransmitters/Receptors/Enzymes/Transporters												
Gene	UniGene Annotation	Accession	Brain Region	Direction	FC Level	Gene Type	Fisher's post-hoc		Scheffe's post-hoc		Fold Changes	
							Sh vs Loh	Sh vs Loh	Sh vs Loh	Sh vs Loh	Sh vs Loh	Sh vs Loh
EST similar to Na ⁺ dependent high affinity glutamate transporter (GLT-1A)	U14005	AK44517	ACC	Down	--	Neurotransmitters/Receptors/Enzymes/Transporters	NS	p<0.05	p<0.05	NS	p<0.05	1.06
Catechol-O-methyltransferase	U14005	M60753	ANG	Down	--	Neurotransmitters/Receptors/Enzymes/Transporters	NS	p<0.05	p<0.05	NS	p<0.05	0.85
AMPA receptor (GluR1)	GluR1 Glutamate receptor ionotropic, AMPA (alpha-1); X17164 RING1R1 Rat non-voltage-gated glutamate receptor, AMPA subunit, GluR1	X17164	LH	Down	--	Neurotransmitters/Receptors/Enzymes/Transporters	NS	p<0.01	p<0.01	NS	p<0.05	1.13
Kainate receptor subunit (KAR)	GluR5 Glutamate receptor ionotropic, kainate 5; X59998	X59998	LH	Down	--	Neurotransmitters/Receptors/Enzymes/Transporters	NS	p<0.01	p<0.01	NS	p<0.05	0.94
G-protein beta-1 subunit (Gbeta1)	Gbeta1 G-protein beta-1 subunit; A027660	A027660	LH	Down	--	Neurotransmitters/Receptors/Enzymes/Transporters	p<0.01	p<0.01	p<0.01	p<0.01	p<0.05	0.51
NMDA receptor subunit 2D (NR2D)	NR2D NMDA receptor subunit 2D; U03260	U03260	LH	Up	++	Neurotransmitters/Receptors/Enzymes/Transporters	p<0.01	p<0.01	p<0.01	p<0.01	p<0.05	2.03
Peripheral benzodiazepine receptor (PBR)	PBR Peripheral benzodiazepine receptor; J05122	J05122	LH	Up	+++	Neurotransmitters/Receptors/Enzymes/Transporters	NS	p<0.05	p<0.05	NS	p<0.099	1.32
beta-Adrenergic receptor kinase	Adrbk1 Adrenergic receptor kinase, beta 1; S48513	S48513	LH	Up	+++	Neurotransmitters/Receptors/Enzymes/Transporters	p<0.05	p<0.01	p<0.01	p<0.05	p<0.078	1.67
Neuron-specific protein (PPE-19)	PPE-19 Neuron-specific protein (PPE-19); M24552	M24552	LH	Down	--	Neurotransmitters/Receptors/Enzymes/Transporters	p<0.01	p<0.01	p<0.01	p<0.01	p<0.05	0.64
Aromatic L-amino acid (Dopa) decarboxylase	Ddc Dopa decarboxylase (aromatic L-amino acid decarboxylase); M18444/M18445 Rat (clone pSHCF-01) liver-specific aromatic L-amino acid decarboxylase gene, exon 1a	M18444	LH	Down	--	Neurotransmitters/Receptors/Enzymes/Transporters	NS	p<0.05	p<0.05	NS	p<0.05	0.96
NMDA receptor-like complex glutamate-NMDA receptor (GluR)	GluR NMDA receptor-like complex glutamate-NMDA receptor; S51973	S51973	LH	Up	++	Neurotransmitters/Receptors/Enzymes/Transporters	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	1.41
alpha 2-adrenergic receptor (RG20)	RG20 alpha 2-adrenergic receptor; M18272	M18272	LH	Up	--	Neurotransmitters/Receptors/Enzymes/Transporters	NS	p<0.01	p<0.01	NS	p<0.01	1.283208
Vesicular inhibitory amino acid transporter (SVANT)	SVANT Vesicular inhibitory amino acid transporter; A099551	A099551	LH	Up	+++	Neurotransmitters/Receptors/Enzymes/Transporters	NS	p<0.01	p<0.01	NS	p<0.01	1.33

Table 1

Gene	UniGene Annotation	Accession	Brain Region	Direction	FC Level	Gene Type	Monoclonal Antibody/ Receptor/Enzyme/ Transcriptase	NS	p<0.01	p<0.05	p<0.05	0.97/0.87	0.45	0.47
Release Machinery														
								Fisher's post-hoc			Scheffe's post-hoc			Fold Changes
								Ctrl vs Sub	Ctrl vs LoA	Sub vs LoA	Ctrl vs Sub	Ctrl vs LoA	Sub vs LoA	Effect
EST184376	ESTs, Moderately similar to SNG1, RAT SYNAPTOTRIN 1 (P29)	AA199879	ANG	Up	-to +	Release Machinery		NS	p<0.05	p<0.05	NS	p<0.083	p<0.074	2.6
Synaptotagmin II	D28512 RATS Rat mRNA for Synaptotagmin II complex	U28512	LH	Up	+++	Release Machinery		NS	p<0.01	p<0.01	NS	p<0.01	p<0.05	1.13
Synaptotagmin XI	transmembrane trafficking protein; syntaxin; intracellular membrane protein with single transmembrane region and two C2-domains	AF004423	LH	Up	++	Release Machinery		p<0.083	p<0.01	p<0.01	NS	p<0.01	p<0.01	1.99
EST, non-processed neuronal beta mRNA		AA955149	LH	Down	--	Release Machinery		p<0.01	p<0.01	p<0.05	p<0.01	NS	NS	0.45
Synaptotagmin VII	Synaptotagmin 3	U20110	VTA	Up	+++	Release Machinery		NS	p<0.01	p<0.01	NS	p<0.01	p<0.01	1.3
														2.71

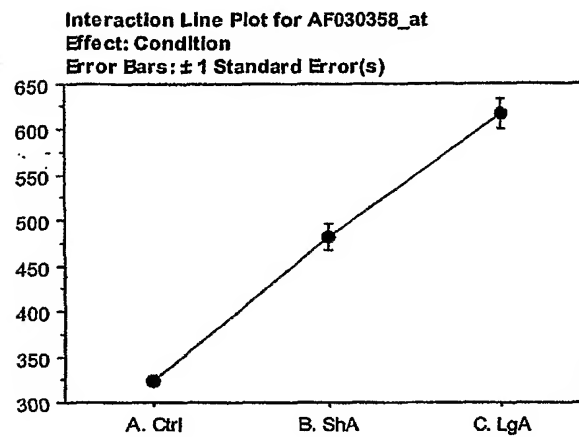
Table 2**GROWTH/STRUCTURE/PLASTICITY GENES****Gene****Average Signal vs Withdrawal Time**Tumor necrosis factor
receptor chain (p60)

Insulin-like growth factor II

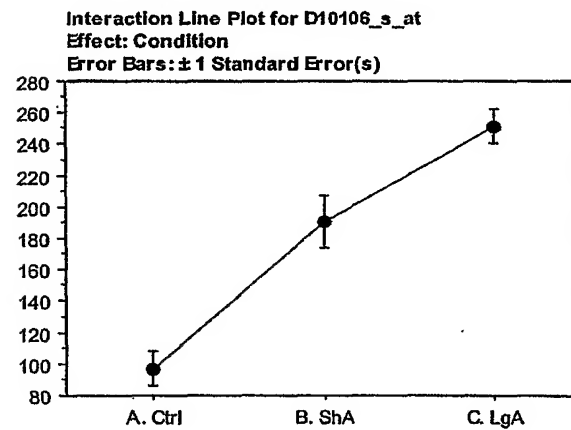


GeneAverage Signal vs Withdrawal Time

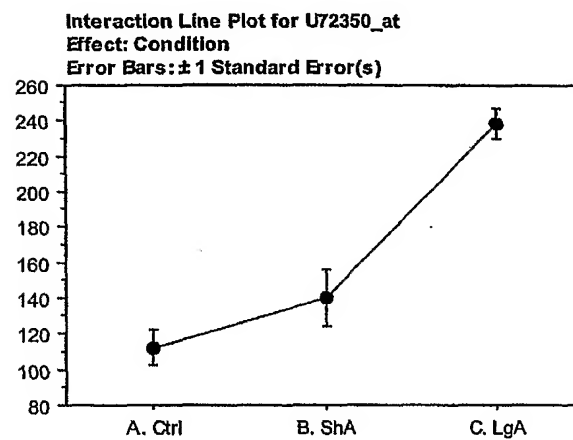
Fractalkine/ Chemokine
CX3C motif ligand 1



Platelet-derived growth
factor A chain (PDGFa)

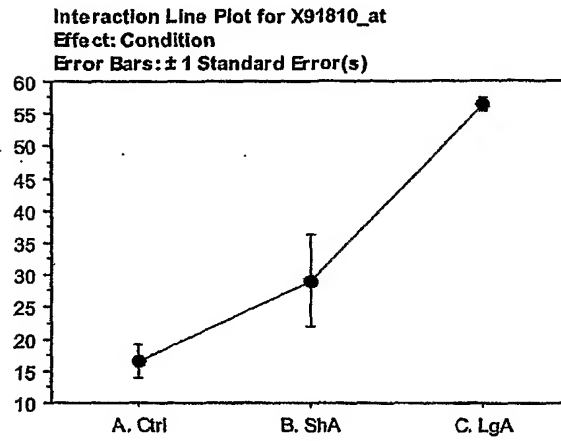


Bcl-x alpha (Bcl-2 family)

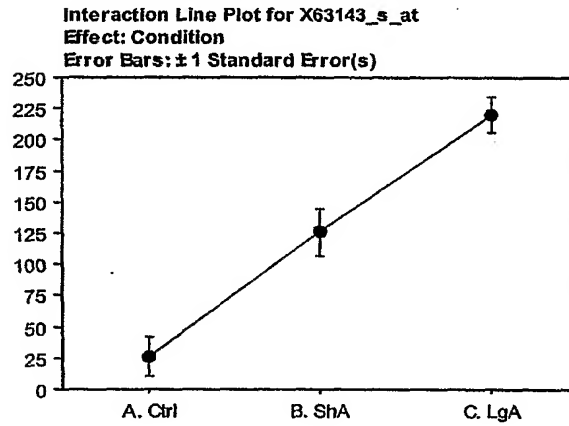


GeneAverage Signal vs Withdrawal Time

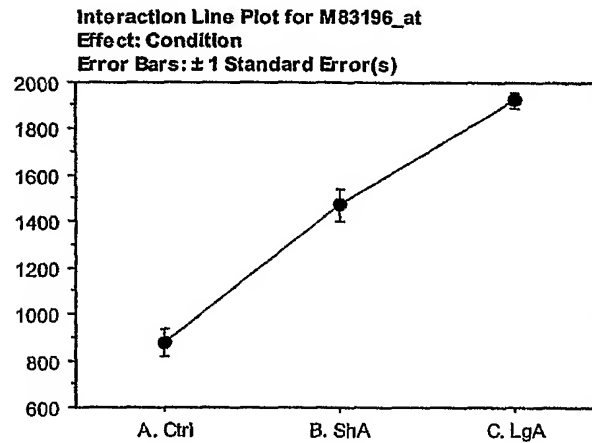
Signal transducer and activator
of transcription 3 (STAT 3)



N-syndecan (Neuroglycan)

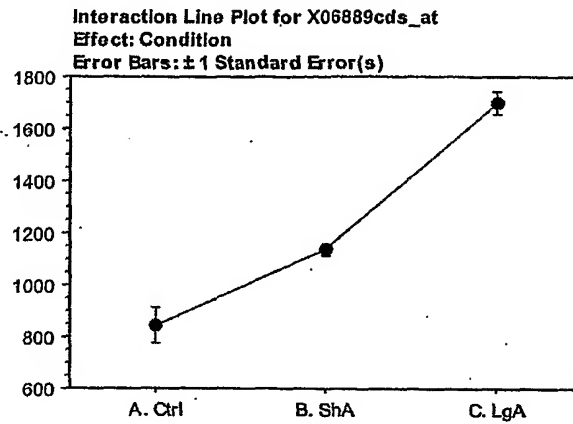


Microtubule-associated
protein (MAP1A)

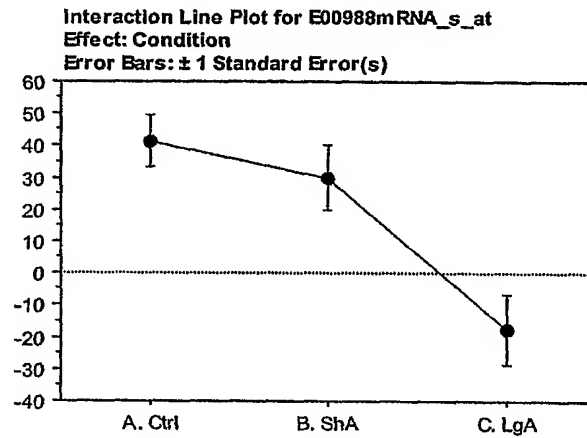
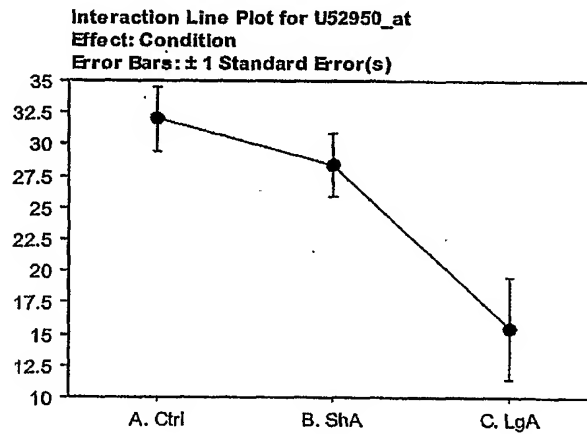


Gene Average Signal vs Withdrawal Time

Ras-related GTPase (Rab3)

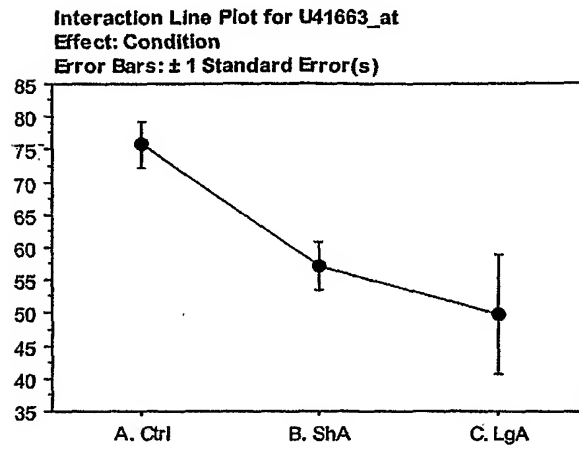


Insulin-like growth factor II

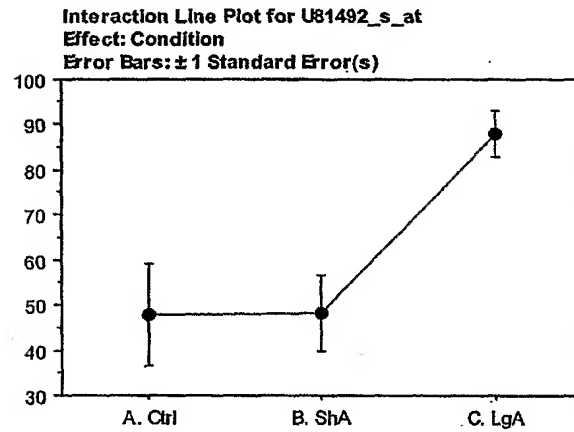
Microtubule-associated
protein (MAP1B)

GeneAverage Signal vs Withdrawal Time

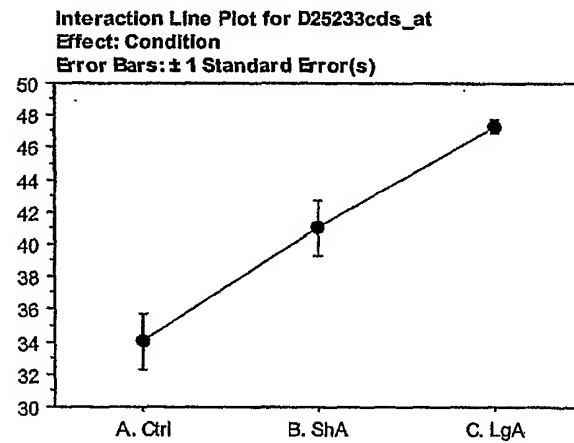
Neurologin 3



Interleukin-3 beta

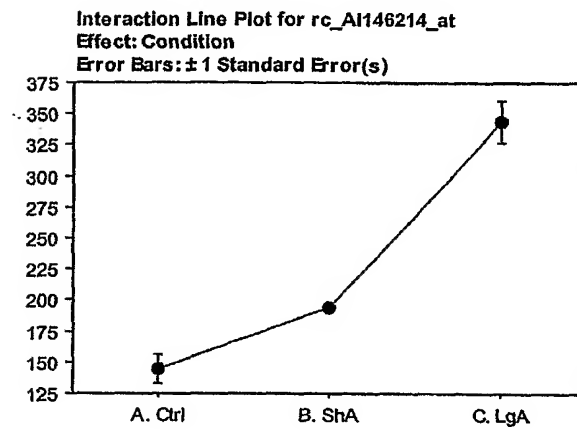


Retinoblastoma protein (pRb)



GeneAverage Signal vs Withdrawal Time

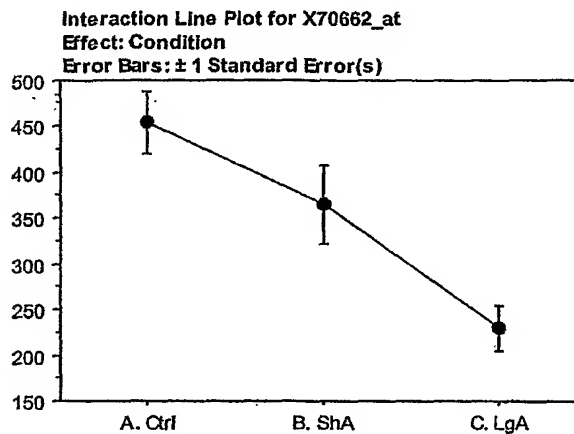
beta S-100 Ca++ binding
protein



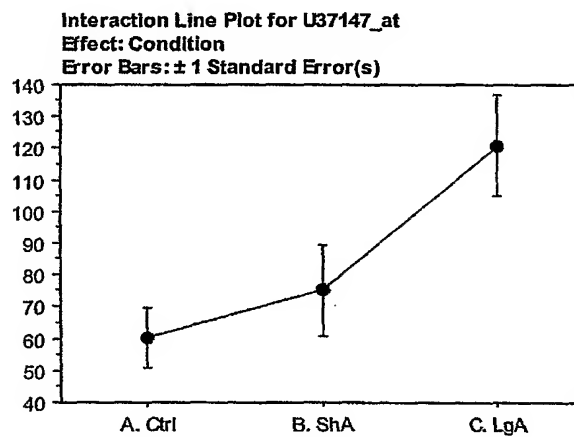
ION PUMPS AND CHANNELS

GeneAverage Signal vs Withdrawal Time

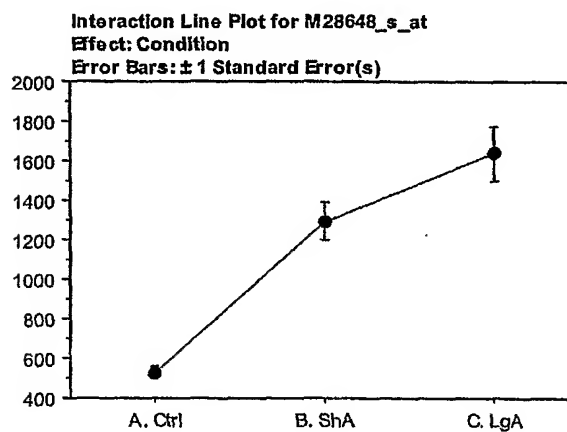
K⁺ channel beta subunit
(Kv1-type)



Na⁺ channel beta 2 subunit
(Scn2b)

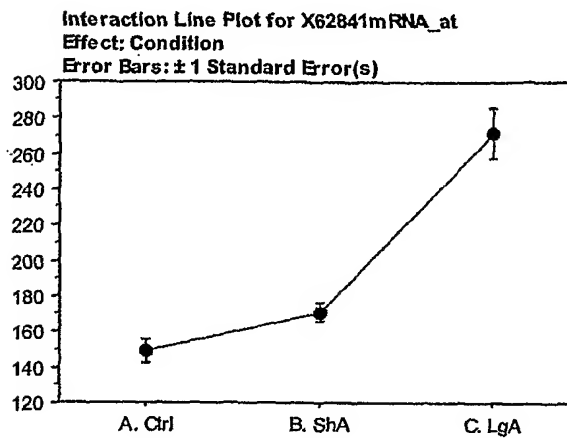


Na⁺,K⁺-transporting ATPase
alpha 2 subunit (Atp1a2)

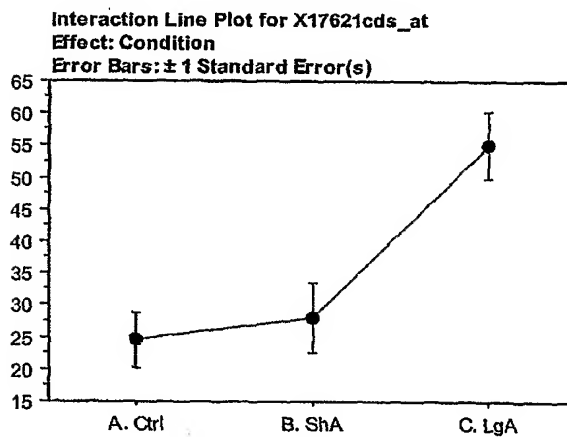


GeneAverage Signal vs Withdrawal Time

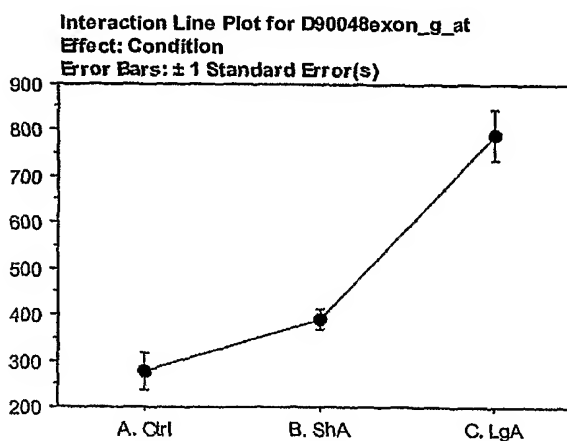
Voltage-gated K⁺ channel
Kv3.4, Shaw-related subfamily,
member 2 (Kcnc2)



K⁺ channel, delayed rectifier
(RCK2)

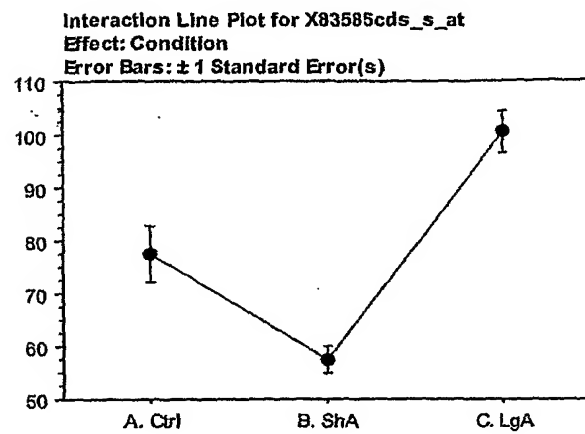


Na⁺,K⁺-ATPase beta 2
subunit

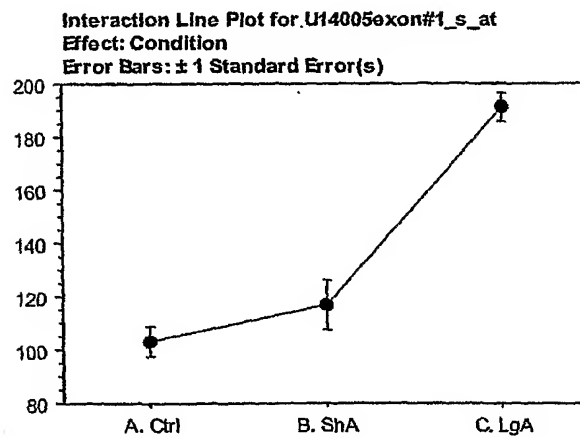


GeneAverage Signal vs Withdrawal Time

K⁺ inward rectifier 10
(Kir4.1)

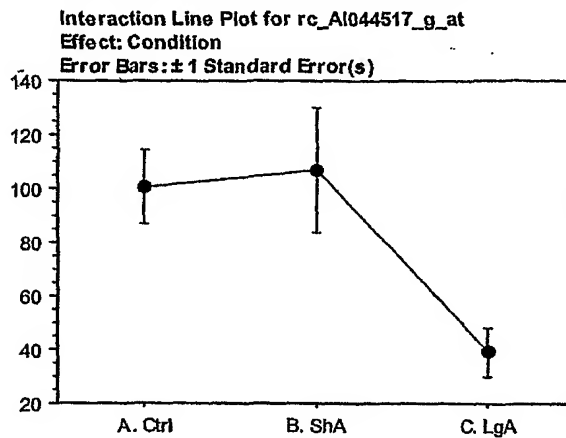


Ca⁺⁺ channel alpha 1
subunit (Cacna1a)

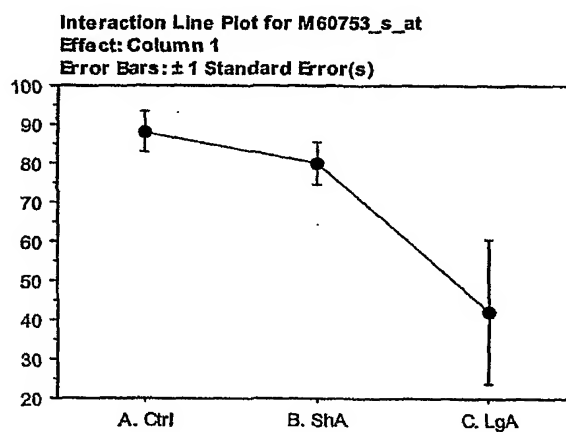


**NEUROTRANSMITTERS/RECEPTORS/
ENZYMES/TRANSPORTERS****Gene****Average Signal vs Withdrawal Time**

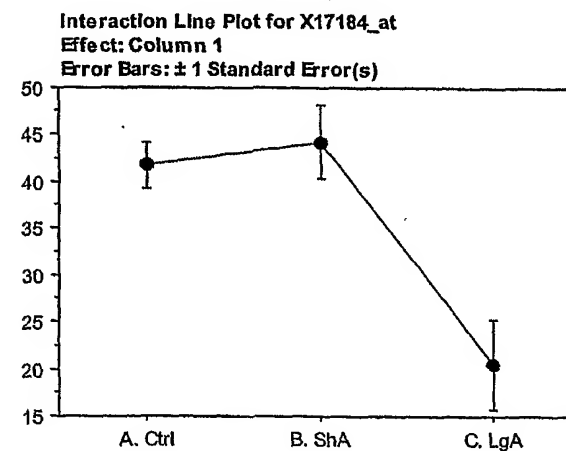
EST similar to Na⁺-dependent
high affinity glutamate
transporter (GLT-1A)



Catechol-O-methyltransferase



AMPA receptor (GluR1)

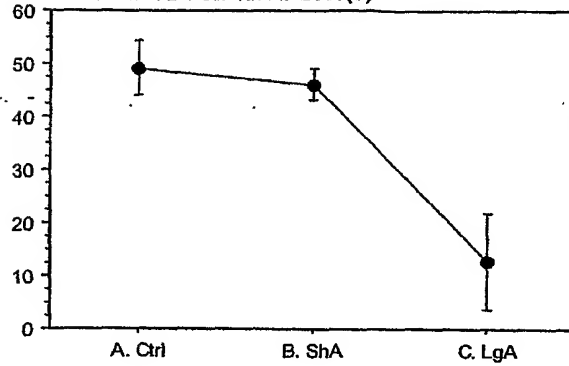


Gene

Average Signal vs Withdrawal Time

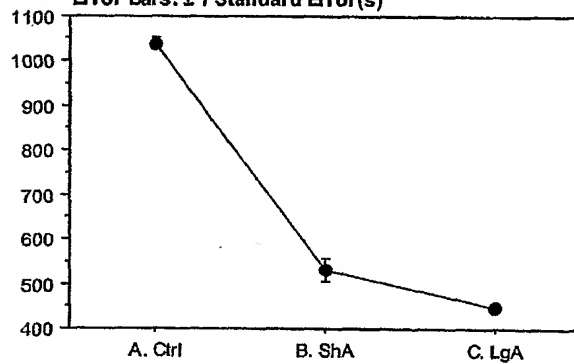
Kainate receptor subunit (KA1)

Interaction Line Plot for X59996mRNA_s_at
Effect: Condition
Error Bars: ± 1 Standard Error(s)



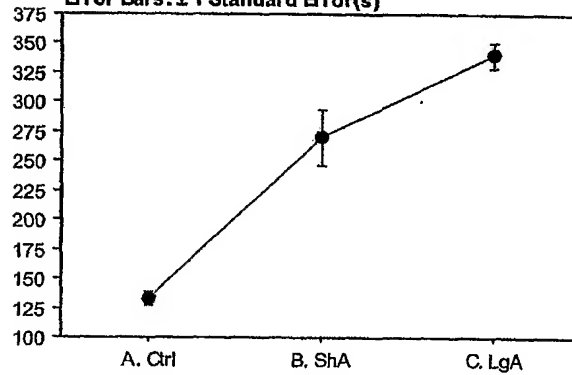
G-protein beta-1 subunit
(r rGbeta1)

Interaction Line Plot for rc_A1227660_s_at
Effect: Condition
Error Bars: ± 1 Standard Error(s)



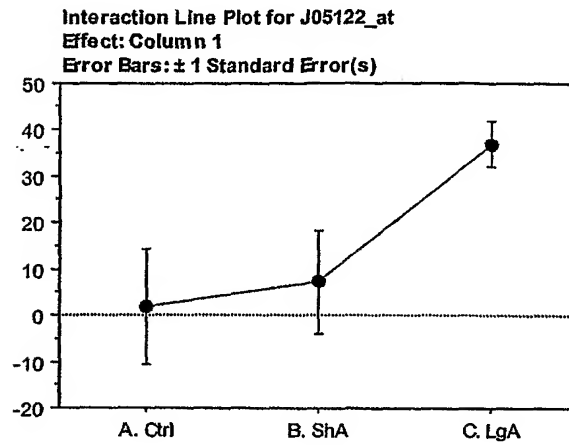
NMDA receptor subunit 2D
(NMDA2D)

Interaction Line Plot for ColuU08260_at
Effect: Condition
Error Bars: ± 1 Standard Error(s)

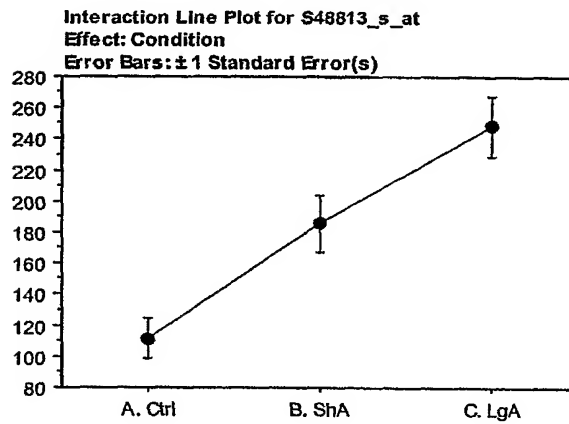


GeneAverage Signal vs Withdrawal Time

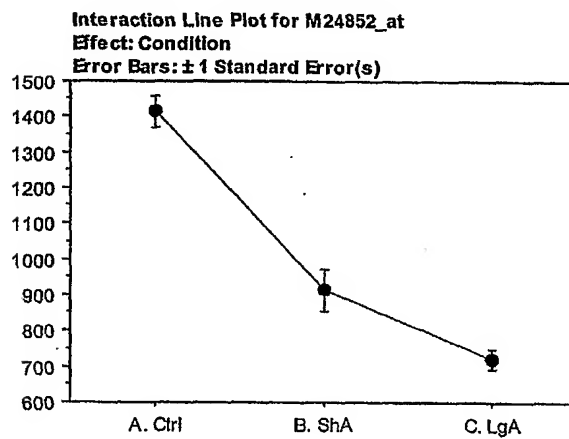
Peripheral benzodiazepine.
receptor (PKBS)



beta-Adrenergic receptor
kinase

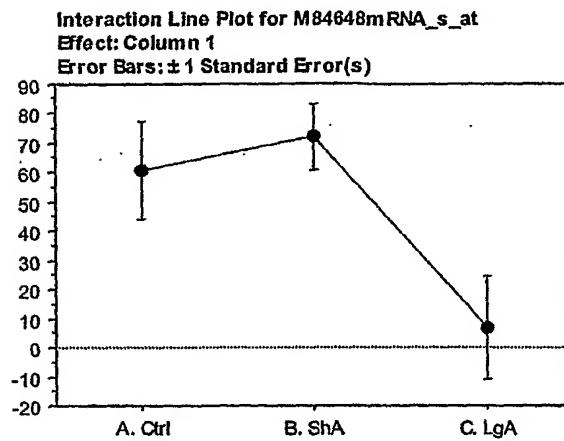


Neuron-specific protein
(PEP-19)

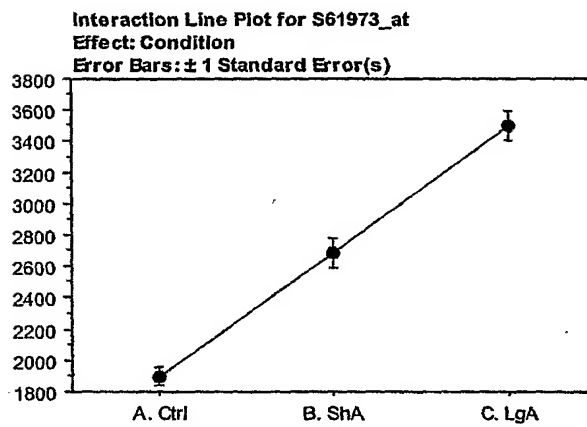


GeneAverage Signal vs Withdrawal Time

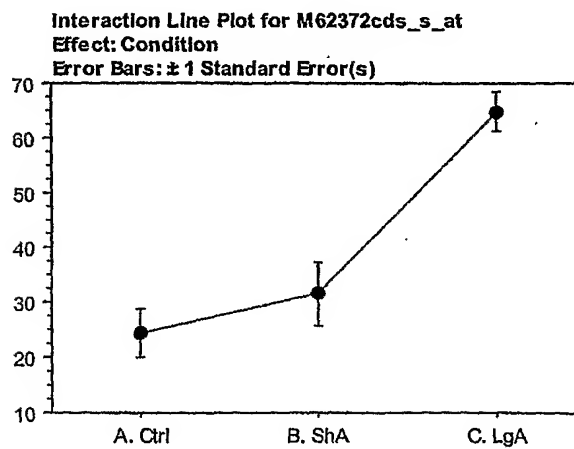
Aromatic L-amino acid
(Ddc Dopa) decarboxylase



NMDA receptor-like complex
glutamate-binding protein(GBP)

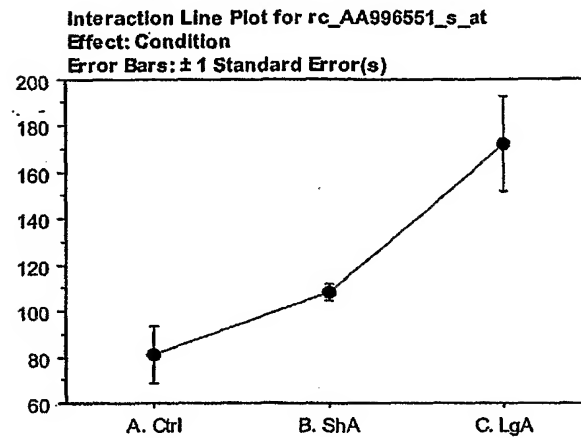


alpha 2-adrenergic receptor
(RG20)

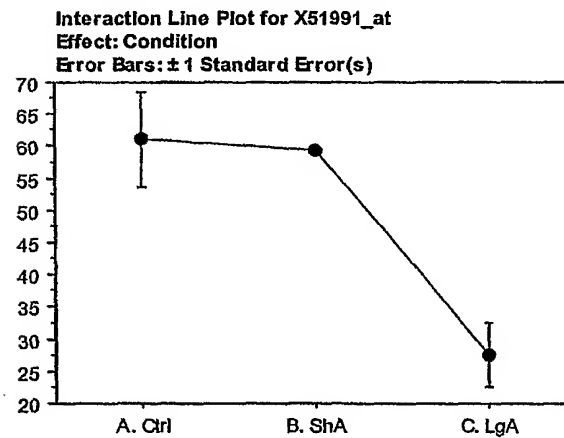


GeneAverage Signal vs Withdrawal Time

Vesicular inhibitory amino
acid transporter (5VIAAT)



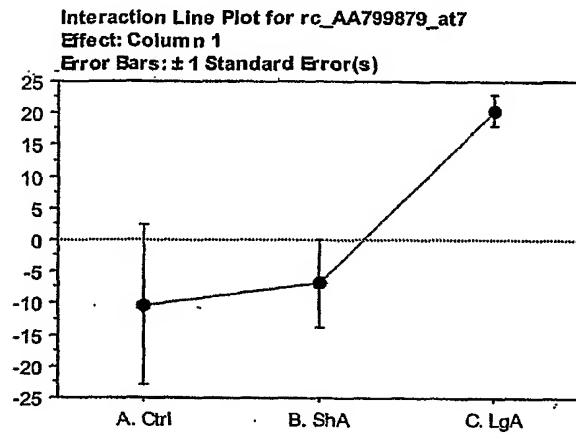
GABAA receptor alpha 3
subunit (Gabra3)



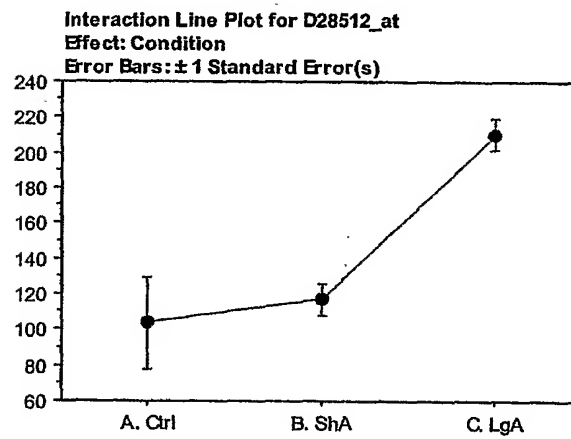
RELEASE MACHINERY

GeneAverage Signal vs Withdrawal Time

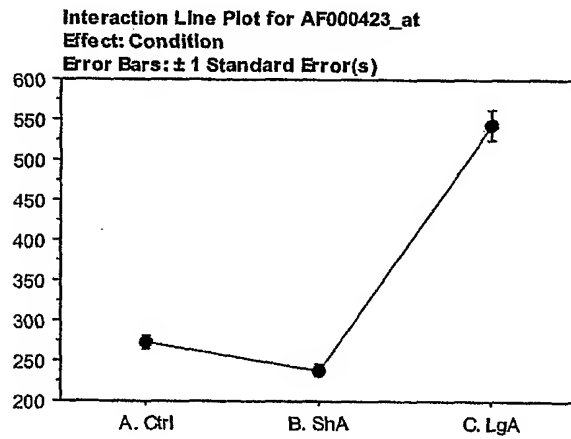
EST189376



Synaptotagmin III

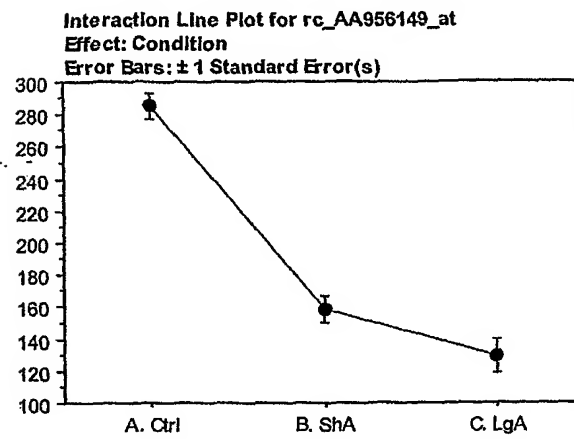


Synaptotagmin XI

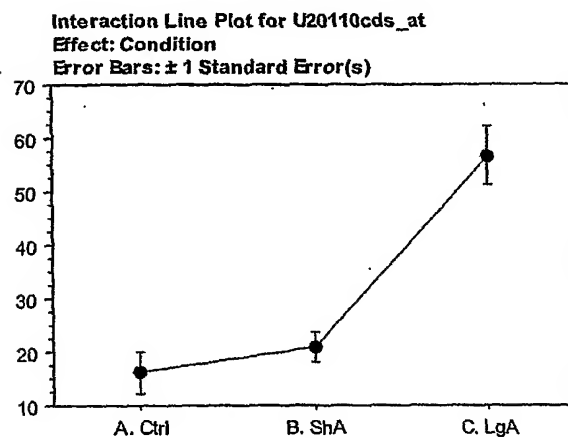


GeneAverage Signal vs Withdrawal Time

EST, non-processed neurexin
I-beta mRNA



Synaptotagmin VIII



Lateral Hypothalamus 230Achip

Probe Set	m	FC MA	FC ME	Gene	Source
1367799_at	NM_012660	2.06	1.10	Statin-like protein	MAS
1367835_at	NM_019279	1.90	1.04	proprotein convertase subtilisin/kexin type 1 inhibitor	MAS
1367868_at	NM_031708	3.79	1.13	adhesion regulating molecule 1	MAS
1367959_a	AF182949	2.98	1.14	sodium channel, voltage-gated, type 1, beta polypeptide	MAS
1368057_at	NM_012804	-1.52	-1.07	ATP-binding cassette, sub-family D (ALD), member 3	MAS
1368082_at	NM_017048	1.94	1.18	Solute carrier family 4, member 2, anion exchange protein 2	MAS
1368359_a	NM_030997	3.31	1.02	VGF nerve growth factor inducible	MAS
1368417_at	NM_019350	1.47	1.40	synaptotagmin 5	MAS
1368425_at	NM_080690	1.64	1.08	cask-interacting protein 1	MAS
1368444_at	NM_022703	1.42	1.38	small glutamine-rich tetratricopeptide repeat (TPR)-containing protein	MAS
1368862_at	NM_033230	1.91	1.17	v-akt murine thymoma viral oncogene homolog 1	MAS
1368951_at	NM_022797	5.67	1.88	glutamate receptor, ionotropic, NMDA2D	MAS
1368959_at	NM_017294	20.72	6.42	protein kinase C and casein kinase substrate in neurons 1	MAS
1369128_at	NM_017262	1.88	1.06	Glutamate receptor, ionotropic, kainate 5	MAS
1369453_at	NM_057136	4.36	1.35	Epsin 1	MAS
1369772_at	AW141210	1.71	1.34	glycine transporter 1	MAS
1369816_at	NM_013018	1.96	1.54	Ras-related small GTP binding protein 3A	MAS
1369926_at	NM_022525	1.60	-1.05	plasma glutathione peroxidase precursor	MAS
1369974_at	NM_012663	2.51	1.62	vesicle-associated membrane protein 2	MAS
1369999_a	NM_053601	1.58	1.11	neuronatin	MAS
1370341_at	AF019973	1.65	1.21	enolase 2, gamma	MAS
1370427_at	L06238	2.17	1.23	Platelet-derived growth factor A chain	MAS
1370519_at	U06069	2.19	1.58	Syntaxin binding protein 1	MAS
1370922_at	L15011	2.47	-1.08	cortixin	MAS
1370938_at	AI535144	1.90	1.16	Rattus norvegicus reg I binding protein I (Rbp1) mRNA, partial cds	MAS
1370964_at	BF283456	1.83	-1.01	arginosuccinate synthetase 1	MAS
1371063_at	AF009603	1.62	1.25	SH3 domain protein 2A	MAS
1371104_at	AF286470	1.82	1.19	—	MAS
1371359_at	BG381670	1.71	1.12	ESTs, Highly similar to MLF2_MOUSE Myeloid leukemia factor 2 (My	MAS
1371528_at	BI274519	2.07	1.11	ESTs, Highly similar to FKB8_MOUSE 38 kDa FK-506 binding protein	MAS
1371578_at	AW915101	1.98	1.44	ESTs	MAS
1371716_at	BE107610	1.40	1.07	ESTs	MAS
1372703_at	BG380680	1.47	1.09	ESTs, Weakly similar to ubiquitin conjugating enzyme [Rattus norveg	MAS
1373470_at	BM388896	-1.44	-1.03	ESTs	MAS
1373787_at	AA943735	1.77	-1.02	glycine transporter 1	MAS
1375149_at	AI145991	2.77	1.08	ESTs, Highly similar to T46266 hypothetical protein DKFZp761A179.1	MAS
1375307_at	BI275772	1.72	1.06	ESTs, Highly similar to RIKEN cDNA 1200013A08 [Mus musculus] [N	MAS
1375612_at	AA965147	-1.97	-1.00	heterogeneous nuclear ribonucleoprotein A1	MAS
1375657_at	BE107438	2.74	-1.10	ESTs	MAS
1375720_at	AI171785	1.65	1.03	ESTs, Highly similar to GBR1_RAT Gamma-aminobutyric acid type B	MAS
1376233_at	AI144891	1.66	1.11	ESTs	MAS
1376345_at	BG381734	1.54	1.04	calcyon; D1 dopamine receptor-interacting protein	MAS
1376904_at	AI716115	2.78	1.08	ESTs	MAS
1382915_at	AI237079	-4.24	-1.02	ESTs	MAS
1383161_a	AI008646	-1.63	-1.29	—	MAS
1386874_at	NM_017151	-1.40	-1.09	ribosomal protein S15	MAS
1386892_at	NM_031975	2.57	1.30	parathymosin	MAS
1386909_a	AF268467	1.65	1.70	voltage-dependent anion channel 1	MAS
1386955_at	BM387903	2.20	1.46	glycoprotein Ib (platelet), beta polypeptide	MAS
1387429_at	NM_012776	2.06	1.20	adrenergic receptor kinase, beta 1	MAS
1388030_a	AF312319	2.64	1.66	gamma-aminobutyric acid (GABA) B receptor, 1	MAS
1388088_a	AB035650	7.42	1.18	transcription factor USF2	MAS
1388158_at	BG057565	1.50	-1.02	HLA-B-associated transcript 1A	MAS
1388309_at	BG378885	1.92	1.22	ESTs	MAS
1388430_at	BI280292	1.54	1.02	ESTs, Highly similar to prostate tumor over expressed gene 1 [Homo	MAS
1389059_at	BI278651	2.63	-1.29	ESTs	MAS
1389240_at	AW527026	1.92	1.21	ESTs	MAS
1389301_at	AI176655	-1.47	-1.09	ESTs	MAS
1390033_at	BG378062	1.79	1.21	ESTs	MAS
1390167_at	BI286834	2.58	-1.07	ESTs	MAS
1390262_a	AI705744	5.01	1.37	ESTs, Weakly similar to nuclear GTPase PIKE [Rattus norvegicus] [R	MAS
1391676_at	AI511097	-2.53	1.03	ESTs	MAS
1367823_at	BF523128	1.90	1.35	tissue inhibitor of metalloproteinase 2	MAS
1389836_a	AI599265	1.38	1.09	Tissue inhibitor of metalloproteinase 3	dChip
1367800_at	NM_013151	1.28	1.20	Plasminogen activator, tissue	dChip
1373672_at	BM384419	-1.22	-1.12	ESTs, Weakly similar to plasminogen activator inhibitor 2 type A [Ratt	dChip

SP	AA848563	1.943548	-1.116183	heat shock 70kD protein 1A	
ACC	AA851136	-1.236515	-1.467442	p21 (CDKN1A)-activated kinase 1	
SP	AA944099	-1.549947	1.259603	platelet derived growth factor receptor, alpha polypeptide	
SP	AB002801	8.777778	2.089947	cyclic nucleotide gated channel alpha 3	
VTA	AB004638	1.275242	2.254279	fibroblast growth factor 18	
VTA	AB013130	1.379007	1.640221	synaptopodin	
SP	AB013890	-2.09417	-1.982063	potassium inwardly-rectifying channel, subfamily J, member 13	
VTA	AB016161	-1.147732	1.032839	gamma-aminobutyric acid (GABA) B receptor, 1	
SP	AF000368	1.294253	-1.095915	sodium channel, voltage-gated, type 9, alpha polypeptide	
VTA	AF000368	1.153766	1.510959	sodium channel, voltage-gated, type 9, alpha polypeptide	
AMY	AF003598	1.364816	1.426526	integrin beta 7	
ACC	AF003825	1.418502	-1.096273	glial cell line derived neurotrophic factor family receptor alpha 2	
AMY	AF007758	1.419998	1.041801	synuclein, alpha	
SP	AF012347	1.989991	1.219064	MAD homolog 9 (Drosophila)	
SP	AF014365	3.825	1.085106	CD44 antigen	
AMY	AF015728	1.240782	1.940325	cyclic nucleotide-gated channel beta subunit 1	
AMY	AF017637	-4.855505	-2.025229	carboxypeptidase Z	
VTA	AF021137	-3.075949	1.490566	potassium inwardly-rectifying channel, subfamily J, member 2	
AMY	AF021935	1.029123	-1.095663	Ser-Thr protein kinase related to the myotonic dystrophy protein kinase	
MPF	AF022083	-1.049755	1.223144	guanine nucleotide binding protein, beta 1	
VTA	AF022083	-1.074567	1.837131	guanine nucleotide binding protein, beta 1	
SP	AF025670	1.020737	1.701665	caspase 6	
VTA	AF027506	-1.709641	-1.470852	solute carrier family 24 (sodium/potassium/calcium exchanger), member 2	
SP	AF028603	1.02444	2.453659	purinergic receptor P2X, ligand-gated ion channel, 2	
AMY	AF030086	-2.072289	2.371429	Rattus norvegicus activity and neurotransmitter-induced early gene 1 (ania-1) mRNA, 3'UTR	
AMY	AF034896	-1.413559	-1.077966	olfactory receptor-like protein	
AMY	AF039218	-1.110863	1.248111	citron	
AMY	AF041246	1.778409	-1.231629	hypocretin receptor 2	
VTA	AF042499	2.070876	3.175889	MAD homolog 7 (Drosophila)	
ACC	AF049239	-1.121527	-1.097757	sodium channel, voltage-gated, type 8, alpha polypeptide	
SP	AF049239	1.011791	1.056272	sodium channel, voltage-gated, type 8, alpha polypeptide	
ACC	AF050659	1.251876	1.093633	Rattus norvegicus activity and neurotransmitter-induced early gene 7 (ania-7) mRNA, 3' UTR	
MPF	AF050659	2.673913	1.356618	Rattus norvegicus activity and neurotransmitter-induced early gene 7 (ania-7) mRNA, 3' UTR	
SP	AF050663	1.058716	-1.115425	Rattus norvegicus activity and neurotransmitter-induced early gene 11 (ania-11) mRNA, 3' UTR	
VTA	AF050664	-1.181034	-1.646552	Rattus norvegicus activity and neurotransmitter-induced early gene 12 (ania-12) mRNA, 3' UTR	
VTA	AF052540	-2.174825	-2.664336	calpain 3	
SP	AF056704	1.898601	1.916471	synapsin 3	
ACC	AF060173	-1.286291	-1.077396	SV2 related protein	
SP	AF065432	2.702422	1.528376	BCL2-like 11 (apoptosis facilitator)	
VTA	AF065432	1.624473	-1.94026	BCL2-like 11 (apoptosis facilitator)	
ACC	AF065433	-1.065119	-1.263503	BCL2-like 11 (apoptosis facilitator)	

MPF	AF065433_at	-1.306889	-1.519833	BCL2-like 11 (apoptosis facilitator)
SP	AF065433_at	1.53501	1.502461	BCL2-like 11 (apoptosis facilitator)
VTA	AF074482_s_at	1.201839	1.248329	G protein-coupled receptor 51
AMY	AF075382_at	-1.246106	-1.990654	cytokine inducible SH2-containing protein 2
MPF	AF075382_at	1.899471	1.365019	cytokine inducible SH2-containing protein 2
MPF	AF081366_at	-1.07729	-1.054648	potassium inwardly-rectifying channel, subfamily J, member 1
ACC	AF090113_at	1.373631	1.007738	glutamate receptor interacting protein 2
MPF	AJ006519_at	1.775159	1.087778	ether-a-go-go-like potassium channel 1
AMY	AJ007632_s_at	1.49322	1.553792	ether-a-go-go-like potassium channel 1
VTA	AJ007632_s_at	1.253138	-1.187813	5-hydroxytryptamine (serotonin) receptor 4
VTA	AJ011370_g_at	1.015038	1.594488	a disintegrin and metalloproteinase domain 17
VTA	AJ012603cds_at	1.00575	1.182545	Bradykinin receptor B1
AMY	AJ132230_at	-2.171212	-2.775758	
AMY	D00698_s_at	-1.148438	-4.429688	glycine receptor, alpha 1 subunit
SP	D00833_at	-1.811414	-1.761787	
SP	D00913_g_at	1.053458	-1.049814	glutamate receptor, ionotropic, N-methyl D-aspartate 2A
ACC	D13211_s_at	-1.561062	-1.097345	glutamate receptor, ionotropic, NMDA2C
VTA	D13212_s_at	1.305048	1.262878	glutamate receptor, ionotropic, NMDA2D
VTA	D13213_s_at	1.182025	1.003468	solute carrier family 2, member 2
AMY	D13962_g_at	1.325854	1.644394	chloride channel, nucleotide-sensitive, 1A
MPF	D13985_at	1.144137	1.275604	
SP	D14478_s_at	-1.822938	1.245614	calpain 8
VTA	D14480_at	-1.463054	-1.625616	sulfolipase, hydroxysteroid preferring 2
VTA	D14987_f_at	1.332506	3.033898	opioid receptor, mu 1
SP	D16349_at	-2.844156	-1.519481	retinoblastoma 1
ACC	D25233UTR#1_at	1.740061	1.431447	cadherin 6
SP	D25290_at	-1.055077	-1.512909	solute carrier family 2, member 5
AMY	D28562_s_at	2.470238	2.084677	
SP	D30781_at	1.689826	1.685644	gamma-aminobutyric acid A receptor, rho 2
AMY	D38494_at	-1.6	-1.027273	gamma-aminobutyric acid A receptor, rho 2
VTA	D38494_at	-1.410714	-2.107143	
SP	D45187_s_at	3.305344	1.273529	
AMY	D49395_s_at	2.312404	1.375228	
SP	D49395_s_at	-2.36646	-1.701863	thymoma viral proto-oncogene 3
SP	D49836_at	1.407767	1.321185	thymoma viral proto-oncogene 3
VTA	D49836_at	-1.362559	-1.414692	GABA receptor rho-3 subunit
VTA	D50671_at	1.031746	-2.061538	phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 1
AMY	D64045_s_at	1.167504	6.453704	ATP-binding cassette, sub-family C (CFTR/MRP), member 9
SP	D83598_at	-1.230915	-1.18496	
SP	D86039_g_at	1.014658	1.011171	phospholipase D2
AMY	D88672_at	-2.093023	-2.727907	
AMY	D90258_s_at	1.455525	1.342944	

AMY	E00988mRNA_s_at	E00988	-2.423188	-2.291787	
ACC	E01789cds_s_at	E01789	1.016399	1.211967	
AMY	E01884cds_s_at	E01884	1.086667	-3.871166	
VTA	E02468cds_s_at	E02468	-1.536842	-2.105263	adrenergic receptor, beta 2
AMY	J03024_at	J03024	1.235897	-2.593361	adrenergic receptor, beta 2
SP	J03024_at	J03024	-1.122807	-2.421053	muscarinic receptor m2
AMY	J03025_at	J03025	1.640288	1.002933	insulin-like growth factor binding protein 2
AMY	J04486_at	J04486	-2.013337	-1.484765	tachikinin receptor 3
SP	J05189_at	J05189	-1.555598	-1.254453	cholinergic receptor, nicotinic, alpha polypeptide 5
MPF	J05231_at	J05231	1.532199	1.152651	
VTA	J05232cds_s_at	J05232	-1.839442	-2.813264	
VTA	K01701_at	K01701	-1.032824	1.221992	protein kinase C, beta 1
ACC	K03486_s_at	K03486	-1.024357	1.087839	interleukin 10
AMY	L02926_s_at	L02926	-2.216216	-1.837838	
ACC	L04739cds_s_at	L04739	-1.149134	1.129425	
VTA	L08492cds_s_at	L08492	-1.720131	-1.540098	
AMY	L08494cds_s_at	L08494	-1.026926	-1.622289	
ACC	L08495cds_s_at	L08495	1.252525	1.027406	
AMY	L08497cds_at	L08497	1.657866	1.393132	
SP	L08497cds_at	L08497	1.400592	-1.249495	B-cell leukemia/lymphoma 2
SP	L14680_g_at	L14680	-1.104756	1.123692	glial cell line derived neurotrophic factor
VTA	L15305_s_at	L15305	-1.277778	1.830508	calcium channel, voltage-dependent, L type, alpha 1E subunit
SP	L15453_at	L15453	-1.681587	-1.351984	heat shock 70kD protein 1A
VTA	L16764_s_at	L16764	1.134927	1.136178	
VTA	L19708_at	L19708	1.591928	-1.405634	prostaglandin-endoperoxide synthase 2
SP	L25925_s_at	L25925	1.144598	1.725982	transforming growth factor, beta receptor 1
ACC	L26110_at	L26110	-1.249791	-1.382623	cholinergic receptor, nicotinic, alpha polypeptide 7
SP	L31619_at	L31619	-2.587209	-3.19186	cholinergic receptor, nicotinic, alpha polypeptide 3
AMY	L31621_s_at	L31621	-3.238806	-2.331343	potassium inwardly-rectifying channel, subfamily J, member 9
SP	L77929_at	L77929	1.439394	-1.136842	tyrosine hydroxylase
AMY	M10244_at	M10244	-1.981224	-1.32898	retinol-binding protein 2
AMY	M13949_at	M13949	1.372549	-1.907143	tachykinin 1
VTA	M15191_s_at	M15191	1.09605	1.498225	cholinergic receptor, nicotinic, alpha polypeptide 4
SP	M15682_at	M15682	1.133276	-1.226859	cholinergic receptor, nicotinic, alpha polypeptide 4
VTA	M15682_at	M15682	1.106005	1.540364	calcium/calmodulin-dependent protein kinase II beta subunit
AMY	M16112_g_at	M16112	-1.424992	-1.16066	guanine nucleotide binding protein, alpha o
AMY	M17526_g_at	M17526	-1.475709	-1.198297	retinol binding protein 1
AMY	M19257_at	M19257	-1.416257	-1.711032	fos-like antigen 1
AMY	M19651_at	M19651	-1.448276	-1.42069	
AMY	M20297_at	M20297	1.707937	-1.424721	sodium channel, voltage-gated, type 2, alpha 1 polypeptide
ACC	M22254_at	M22254	1.244326	1.400619	potassium voltage-gated channel, Isk-related subfamily, member 1
AMY	M22412_at	M22412	1.065217	2.202247	interleukin 2

AMY	M22899_at	M22899	-6.512195	-3.365854	interleukin 2	
VTA	M22899_at	M22899	1.111111	2.222222	muscarinic acetylcholine receptor M5	
SP	M22926mRNA_at	M22926	1.032065	1.487079	somatostatin	
AMY	M25890_at	M25890	1.003148	-1.319558		
AMY	M26745cds_s_at	M26745	-1.680851	-1.170213		
SP	M26745cds_s_at	M26745	1.644737	1.644737		
MPF	M27223_at	M27223	-1.56162	-1.140845	insulin-like growth factor 1 receptor	
SP	M27293_s_at	M27293	1.449536	1.036299	cytochrome oxidase subunit Vlc	
AMY	M27466_at	M27466	1.06253	1.136854	complement component 3	
AMY	M29866_s_at	M29866	-2.487414	-3.805492	complement component 3	
MPF	M29866_s_at	M29866	-1.902098	-12.11888	complement component 3	
SP	M29866_s_at	M29866	1.050366	1.023194	transforming growth factor alpha	
AMY	M30312cds_s_at	M30312	-2.597107	-1.280992		
AMY	M31076_at	M31076	-1.479487	-1.378846		
ACC	M31433mRNA#1_at	M31433	1.055556	-1.135711		
MPF	M31433mRNA#1_at	M31433	-2.049704	-1.598817	insulin-like growth factor binding protein 3	
AMY	M31837_at	M31837	-1.792683	-2.33689	dopamine receptor 1A	
AMY	M35077_s_at	M35077	1.569207	1.637937	dopamine receptor 1A	
SP	M35077_s_at	M35077	-1.239796	-1.294218	interleukin 2 receptor, beta chain	
SP	M55050_at	M55050	1.468104	1.185776	glycine receptor, alpha 3	
AMY	M55250_at	M55250	-2.022989	-3.390805	neurotrophic tyrosine kinase, receptor, type 2	
SP	M55293_at	M55293	2.30829	1.528302	calcium channel, voltage-dependent, L type, alpha 1D subunit	
AMY	M57682_at	M57682	-3.555556	-6.981481	potassium voltage gated channel, Shaw-related subfamily, member 2	
SP	M59313_at	M59313	-10.44444	-7.388889	potassium voltage gated channel, Shal-related family, member 2	
AMY	M59980_s_at	M59980	-1.479148	-1.01712	adrenergic receptor, alpha 1d	
AMY	M60654_at	M60654	-1.742489	-2.334764		
AMY	M62372cds_s_at	M62372	-1.082653	2.217195	5-hydroxytryptamine (serotonin) receptor 2A	
AMY	M64867_at	M64867	-1.123096	-1.085025	transforming growth factor, beta receptor 3	
MPF	M77809_at	M77809	-1.96	-2.061667	transforming growth factor, beta receptor 3	
VTA	M80784_s_at	M80784	1.416778	1.713366	GABA-alpha receptor gamma-3 subunit	
VTA	M81142_s_at	M81142	-3.006623	-2.721854		
AMY	M82824_s_at	M82824	-1.192422	1.138748	dopamine receptor 4	
VTA	M84009_at	M84009	-1.88563	-3.721408	calcium channel, voltage-dependent, alpha2/delta subunit 1	
SP	M86621_at	M86621	-1.534023	-1.484962		
AMY	M86742cds_s_at	M86742	-1.186047	-1.327696	ATPase, H+/K+ transporting, nongastric, alpha polypeptide	
VTA	M90398_at	M90398	-1.180763	-1.043118	adenosine A2B receptor	
MPF	M91466_at	M91466	-1.216733	-1.569721		
AMY	M91595exon_s_at	M91595	-2.45737	-1.719755		
AMY	M91599mRNA_g_at	M91599	1.097087	-1.076696		
VTA	M91599mRNA_g_at	M91599	1.031686	1.11967	calcium channel, voltage-dependent, N type, alpha 1B subunit	
ACC	M92905_s_at	M92905	-1.029584	-1.236192	solute carrier family 6 (neurotransmitter transporter, GABA), member 13	
AMY	M95762_at	M95762	-1.825235	-1.236158	neurexin 1	

ACC	M96375 s at	M96375	-1.097785	1.356584	myelin oligodendrocyte glycoprotein	
AMY	M99485 at	M99485	1.41779	-1.118346	phosphodiesterase 4B	
VTA	rc AA799729 at	AA799729	1.051724	-1.745902	Rattus norvegicus transcribed sequence with moderate similarity to protein sp:O43759 (H.sapiens)	
AMY	rc AA799879 at	AA799879	1.924747	2.227806	heat shock 70kD protein 1B	
VTA	rc AA818604 s at	AA818604	1.670036	1.520845	Ras-related GTP-binding protein Rab29	
SP	rc AA858977 at	AA858977	-1.935484	-2.370968	Ras-related GTP-binding protein Rab29	
VTA	rc AA858977 at	AA858977	5.548673	1.583333		
VTA	rc AA893870 g at	AA893870	-1.000483	-2.301714		
SP	rc AA894087 at	AA894087	-1.113038	-1.12799	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	
AMY	rc AA900476 g at	AA900476	-1.278149	-1.637681	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	
SP	rc AA900476 g at	AA900476	-1.241925	-1.735275	synixin binding protein Munc18-2	
VTA	rc AA964359 s at	AA964359	-1.305556	1.434263	heat shock 27kDa protein 1	
VTA	rc AA998683 g at	AA998683	1.127888	1.530233	leptin receptor	
ACC	rc AA998983 at	AA998983	1.097345	2.384615	macrophage migration inhibitory factor	
ACC	rc AI009801 at	AI009801	-1.115271	-1.080384	glutamine synthetase 1	
AMY	rc AI012265 i at	AI012265	1.452669	1.31535	insulin-like growth factor-binding protein 5	
MPF	rc AI029920 s at	AI029920	-1.414406	1.01616	insulin-like growth factor-binding protein 5	
VTA	rc AI029920 s at	AI029920	-1.572693	-1.533197	brain derived neurotrophic factor	
MPF	rc AI030286 s at	AI030286	1.307363	1.634904	nestin	
AMY	rc AI030685 s at	AI030685	1.064615	-1.634393	solute carrier family 1, member 2	
ACC	rc AI044517 g at	AI044517	-1.90683	-1.959218	dopa decarboxylase	
MPF	rc AI044610 s at	AI044610	-1.351994	-1.56823	neuronal pentraxin receptor	
ACC	rc AI045501 s at	AI045501	1.246668	1.160039	neuronal pentraxin receptor	
SP	rc AI045501 s at	AI045501	-1.084203	-1.049448		
VTA	rc AI137657 at	AI137657	1.571429	-2.194805	neurabin 1	
MPF	rc AI145444 at	AI145444	-1.773131	-1.07858		
VTA	rc AI146214 at	AI146214	1.833937	1.620061	suppression of tumorigenicity 13 (colon carcinoma) Hsp70-interacting protein	
AMY	rc AI171166 at	AI171166	1.535167	-1.03233	suppression of tumorigenicity 13 (colon carcinoma) Hsp70-interacting protein	
SP	rc AI171166 at	AI171166	-1.074247	-1.589304	heat shock 27kDa protein 1	
VTA	rc AI176658 s at	AI176658	1.071454	1.601921	guanine nucleotide binding protein, beta 1	
VTA	rc AI227660 s at	AI227660	1.465583	1.483917	Rattus norvegicus hypothetical gene supported by NM_013066 (LOC360449), mRNA	
ACC	rc AI228850 s at	AI228850	-1.268522	-1.170288	guanine nucleotide binding protein, beta 1	
VTA	rc AI230404 s at	AI230404	1.511462	1.753015	fos-like antigen 2	
AMY	rc AI230842 at	AI230842	1.772426	1.778852	stress activated protein kinase alpha II	
ACC	rc AI231354 at	AI231354	-1.435148	-1.269825	stress activated protein kinase alpha II	
VTA	rc AI231354 g at	AI231354	-1.277972	1.141147	glutathione S-transferase, alpha 1	
AMY	rc AI235747 at	AI235747	1.096014	-1.404132		
AMY	S37461 f at	S37461	-1.106762	-2.124555		
VTA	S37461 f at	S37461	-2.819444	-1.736111		
AMY	S42358 s at	S42358	1.599325	1.056807		
SP	S47609 s at	S47609	1.416535	1.205197		
VTA	S53987 at	S53987	2.082759	1.568831		

AMY	S54212_at	S54212	-1.149194	1.04065	
SP	S56481_s_at	S56481	1.412098	1.313599	
AMY	S59525_s_at	S59525	-1.747283	-1.592391	
SP	S59525_s_at	S59525	-2.636364	-1.690909	
ACC	S61973_g_at	S61973	-1.068792	-1.001844	
SP	S62933_i_at	S62933	4.761194	-1.250784	
SP	S66024_g_at	S66024	1.495652	1.524505	
AMY	S68944_l_at	S68944	-1.283297	1.64858	nitric oxide synthase 2, inducible
AMY	S71597_s_at	S71597	2.052459	1.916327	
ACC	S72505_f_at	S72505	1.314892	1.442016	
VTA	S77863_s_at	S77863	1.476378	-1.024889	
VTA	S78154_at	S78154	-1.570881	-1.46798	
ACC	S79676_s_at	S79676	1.573875	1.454669	
VTA	S79903mRNA_at	S79903	-1.609047	-2.927302	opioid receptor, delta 1
SP	U00475_at	U00475	-1.6125	-1.4125	prostaglandin-endoperoxide synthase 1
ACC	U03388_s_at	U03388	1.326597	-1.057282	tumor necrosis factor (ligand) superfamily, member 6
VTA	U03470_at	U03470	-1.829268	-1.420732	transforming growth factor, beta 3
AMY	U03491_at	U03491	-1.270622	-1.305355	transforming growth factor, beta 3
SP	U03491_at	U03491	1.143872	1.05401	transforming growth factor, beta 3
AMY	U03491_g_at	U03491	-1.332904	-1.37311	glutamate receptor, ionotropic, kainate 4
SP	U08257_at	U08257	-1.626845	-1.420664	glutamate receptor, ionotropic, NMDA2D
ACC	U08260_at	U08260	1.433073	1.233501	contactin 3
MPF	U11031_at	U11031	-1.226122	1.730226	
VTA	U16359cds_at	U16359	-1.929929	-3.06057	Huntington disease gene homolog
VTA	U18650_at	U18650	1.112827	1.076795	5-hydroxytryptamine (serotonin) receptor 4
SP	U20907_at	U20907	1.406844	4.285714	5-hydroxytryptamine (serotonin) receptor 4
VTA	U20907_at	U20907	2.306569	-1.514768	Eph receptor A7
VTA	U21954_at	U21954	-1.247423	-3.020619	purinergic receptor P2Y, G-protein coupled 1
SP	U22830_at	U22830	-2.062992	-1.641732	purinergic receptor P2Y, G-protein coupled 1
VTA	U22830_at	U22830	1.378685	-1.123355	potassium inwardly-rectifying channel, subfamily J, member 10
ACC	U27558_at	U27558	1.26815	1.229943	glutamate receptor, ionotropic, N-methyl-D-aspartate 3A
AMY	U29873_at	U29873	-1.460894	-1.061453	microtubule-associated protein 2
ACC	U30938_at	U30938	1.292985	1.103875	calcium channel, voltage-dependent, alpha 1C subunit
SP	U31815_s_at	U31815	-2.529274	-1.077283	5-hydroxytryptamine (serotonin) receptor 2C
AMY	U35315_at	U35315	1.646552	1.281879	brevican
ACC	U37142_at	U37142	-1.078302	1.034517	
ACC	U37147_at	U37147	-1.411871	-1.080709	neurologin 2
AMY	U41662_at	U41662	-1.328488	-1.386628	complement component 4a
AMY	U42719_at	U42719	-1.814574	-1.187541	interleukin 1 receptor-like 2
SP	U49066_at	U49066	-2.70028	-1.37535	caspase 3
SP	U49930_at	U49930	-1.270548	-1.40411	phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 1
SP	U50412_at	U50412	-1.024514	-1.131868	phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 1

VTA	U50412_at	U50412	1.172331	1.926841	solute carrier family 7, member 3	
AMY	U53927_at	U53927	-3.135385	-1.409231	estrogen receptor 2	
SP	U57439_g_at	U57439	1.315892	1.723785	metabotropic glutamate receptor 8	
AMY	U63288_at	U63288	1.121807	1.026056	interleukin 15	
SP	U69272_g_at	U69272	-2.932203	-4.983051	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 3	
ACC	U69884_at	U69884	-1.192277	-1.157683	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 3	
AMY	U69884_at	U69884	1.009804	-1.724919	mitogen activated protein kinase 14	
VTA	U73142_at	U73142	1.10984	1.678201		
VTA	U75899mRNA_at	U75899	-1.191489	-1.462006	cyclic nucleotide-gated cation channel	
SP	U76220_at	U76220	1.423913	1.984848	sodium channel, voltage-gated, type 9, alpha polypeptide	
MPF	U79568_s_at	U79568	2.407925	1.407357	interleukin 3	
MPF	U81492_s_at	U81492	-1.439383	1.933239	forkhead box M1	
ACC	U83112_at	U83112	1.020257	1.168978	guanine nucleotide binding protein, beta 1	
VTA	U88324_at	U88324	1.353066	1.40972	guanine nucleotide binding protein, beta 1	
VTA	U88324_g_at	U88324	1.475683	1.540453	calpain 9 (nCL-4)	
SP	U89514_at	U89514	3.348548	-1.04461	solute carrier family 1 (high affinity aspartate/glutamate transporter), member 6	
VTA	U89608_at	U89608	1.745418	2.934932	potassium voltage-gated channel, KQT-like subfamily, member 1	
VTA	U92655_at	U92655	-1.203704	-1.62963	CC-chemokine-binding receptor JAB61	
AMY	U92803_at	U92803	1.297203	1.570703		
AMY	X03347cds_g_at	X03347	-1.100416	-1.004859	insulin-like growth factor 1	
VTA	X06107_r_at	X06107	-2.167262	-2.042173	RAB3A, member RAS oncogene family	
ACC	X06889cds_at	X06889	1.198613	1.195181		
AMY	X06890cds_at	X06890	1.53932	1.253201		
ACC	X15466cds_at	X15466	-1.13948	-1.11323		
AMY	X16002cds_s_at	X16002	-1.067149	1.104727	insulin-like growth factor 2	
SP	X16703_i_at	X16703	-6.391304	-7.434783	insulin-like growth factor 2	
VTA	X16703_i_at	X16703	1.813084	8.818182	insulin-like growth factor 2	
AMY	X16703_r_at	X16703	1.102684	-1.22963	insulin-like growth factor 2	
SP	X16703_r_at	X16703	1.921739	1.268293		
AMY	X17012mRNA_s_at	X17012	-2.904584	-1.653319	glutamate receptor, ionotropic, AMPA1 (alpha 1)	
AMY	X17184_at	X17184	-1.504791	-1.375871		
ACC	X17621cds_at	X17621	1.03678	1.010142	gamma-aminobutyric acid receptor, subunit alpha 3	
SP	X51991_at	X51991	-1.234447	1.000514	complement component 3	
VTA	X52477_at	X52477	-1.399766	-1.783168	glycogen synthase kinase 3 beta	
MPF	X53428cds_s_at	X53428	1.492795	1.171946	glycine receptor, alpha 2 subunit	
AMY	X57281_at	X57281	1.424747	1.1037		
AMY	X57659_at	X57659	-1.107223	1.046893	interleukin 10	
AMY	X60675_at	X60675	-1.284543	-1.350117	vimentin	
AMY	X62952_at	X62952	-2.087663	-1.376186	solute carrier family 1, member 3	
AMY	X63744_at	X63744	-1.071485	1.825961	neuronal d4 domain family member	
AMY	X66022mRNA#3_i_at	X66022	1.247934	1.365633	interleukin 4 receptor	
AMY	X69903_at	X69903	-1.263048	-1.103862	interleukin 4 receptor	

VTA	X69903_at	X69903	1.369725	1.74716	potassium voltage gated channel, shaker related subfamily, beta member 1	
ACC	X70662_at	X70662	1.142429	-1.018916	glycogen synthase kinase 3 beta	
SP	X73653_at	X73653	-3.690647	-4.043165	inositol 1,4,5-trisphosphate 3-kinase B	
VTA	X74227cds_at	X74227	-1.087664	1.224293	cholinergic receptor, nicotinic, delta polypeptide	
ACC	X74835cds_at	X74835	-1.029364	-1.050571	cholinergic receptor, nicotinic, epsilon polypeptide	
SP	X74836cds_s_at	X74836	-1.090328	1.597668	sodium channel, nonvoltage-gated 1 gamma	
SP	X77933_at	X77933	-2.274933	2.005405	synuclein, gamma	
SP	X86789_at	X86789	-1.34827	-1.481366	potassium inwardly-rectifying channel, subfamily J, member 4	
VTA	X87635_at	X87635	-2.175701	-1.947664	sodium channel, voltage-gated, type 10, alpha polypeptide	
VTA	X92184_at	X92184	1.299413	3.772727	purinergic receptor P2X, ligand-gated ion channel, 7	
ACC	X95882_at	X95882	1.623301	-1.061005	presenilin-2	
ACC	X99267_g_at	X99267	1.183964	1.023542	superoxide dismutase 2	
SP	Y00497_s_at	Y00497	1.112662	-1.433646	glutamate receptor, ionotropic, kainate 2	
ACC	Z11548_at	Z11548	1.485304	1.224876	glutamate receptor, ionotropic, kainate 2	
SP	Z11548_at	Z11548	-1.120169	-1.130228	POU domain, class 3, transcription factor 4	
AMY	Z11834_at	Z11834	-1.403939	-1.075674		
VTA	Z11932cds_g_at	Z11932	-1.34488	1.854749	platelet derived growth factor, alpha	
SP	Z14120cds_s_at	Z14120	1.404895	1.08524	cAMP responsive element modulator	
SP	Z15158mRNA_at	Z15158	-2.92	-2.38		
AMY	Z38067exon_at	Z38067	1.437715	-1.059766	met proto-oncogene	
ACC	Z46374cds_s_at	Z46374	1.838655	1.404365		
VTA	Z49748exon_at	Z49748	-1.454545	-1.325329	chloride channel 5	
MPF	Z56277_i_at	Z56277	-1.61746	1.031097	potassium voltage-gated channel, subfamily H (eag-related), member 2	
VTA	Z96106_at	Z96106	1.231948	1.795661		

Claims

1. A method for treating opioid drug addiction of a patient comprising:
administering to the patient, a pharmaceutical agent having a beneficial
5 interaction with any one or more of
- a) a signaling molecule selected from the group consisting of
insulin-like growth factor II, interleukin-3 (IL-3), interleukin-3
beta, fractalkine/chemokine CX3C motif ligand 1, platelet derived
10 growth factor A chain, Neuroligin 3, neuron-specific protein
(PEP-19), Synaptamin XI;
 - b) an enzyme selected from the group consisting of catechol-O-
methyltransferase, beta-adrenergic receptor kinase, Ras-related
GTPase, Ras-related GTPase beta S-100, aromatic L-amino acid
15 decarboxylase, beta adrenergic receptor kinase, Synaptotagmin
VIII, G-protein beta-1 subunit;
 - c) an ion channel selected from the group consisting of potassium
channel beta subunits, sodium channel beta 2 subunit, voltage
gated potassium channel Kv3.4, Saw-related subfamily member
2, potassium channel delayed rectifier, potassium inward rectifier
20 10 (Kir 4.1), calcium channel alpha 1 subunit;
 - d) a receptor selected from the group consisting of AMPA receptor
GluR1, Kainate receptor KA1, Peripheral benzodiazepine
receptor, alpha 2-adrenergic receptor, NMDA receptor-like
complex glutamate binding protein, GABBA receptor alpha 3
25 subunit, tumor necrosis factor receptor chain, NMDA receptor
subunit 2D, non-processes neurexin1-beta mRNA,;
 - e) a receptor coupling protein;
 - f) a transporter selected from the group consisting of vesicular
inhibitory amino acid transporter and sodium dependent high
30 affinity glutamate transporter, sodium or potassium ion
transporting ATPase alpha 2 subunit;
 - g). a protein from EST AA799879 or AA956149; and
 - h). a growth, survival, functional, structural protein selected from the
group consisting of Bcl-x alpha, signal transducer and activator of

transcription 3 (STAT3), Retinoblastoma protein, Nsyndecan (syndecan-3 or Neuroglycan), EST189376, Synaptotagmin VIII, Calcium ion binding protein, Microtubule-associated protein (MAP1A).

5

2. A method according to claim 1 wherein the beneficial interaction is any one of agonism, antagonism, inhibition, activation, blockage.

3. A method according to claim 2 wherein the beneficial action is agonism, antagonism, mimicry or antimimicry with a signaling molecule.

10

4. A method according to claim 2 wherein the beneficial action is inhibition, blockage or activation with an enzyme.

5. A method according to claim 2 wherein the beneficial action is blockage or activation with an ion channel.

15

6. A method according to claim 2 wherein the beneficial action is agonism or antagonism with a receptor.

20

7. A method according to claim 2 wherein the beneficial action is activation, inhibition, agonism, or antagonism with a receptor coupling protein.

8. A method according to claim 2 wherein the beneficial action is activation or inhibition with a transporter molecule.

25

9. A method for screening for an interactive pharmaceutical agent comprising:

combining a potential pharmaceutical agent, a label entity and a gene product selected from the group consisting of a signaling molecule, an enzyme, an ion channel, a receptor, a receptor coupling protein, a transporter molecule and a growth/survival/functional/structural protein, wherein the label entity is converted to a detectable label when the candidate chemical entity beneficially interacts with the gene product, and

30

detecting the presence and/or quantity of detectable label present wherein a positive detection indicates that the potential pharmaceutical agent is an interactive pharmaceutical agent.

5 10. A method according to claim 9 wherein the combining step is an *in vitro* process.

11. A method according to claim 9 wherein the combining step is an *in vivo* process.

10

12. A method to identify genes comprising comparing expression of an mRNA obtained from a drug addicted animal to expression of the mRNA obtained from a non-drug addicted animal, wherein an increase or decrease in expression of the mRNA obtained from the drug addicted animal relative to
15 expression of the mRNA obtained from the non-drug addicted animal indicates that expression of the mRNA is modulated in response to drug addiction.

1/4

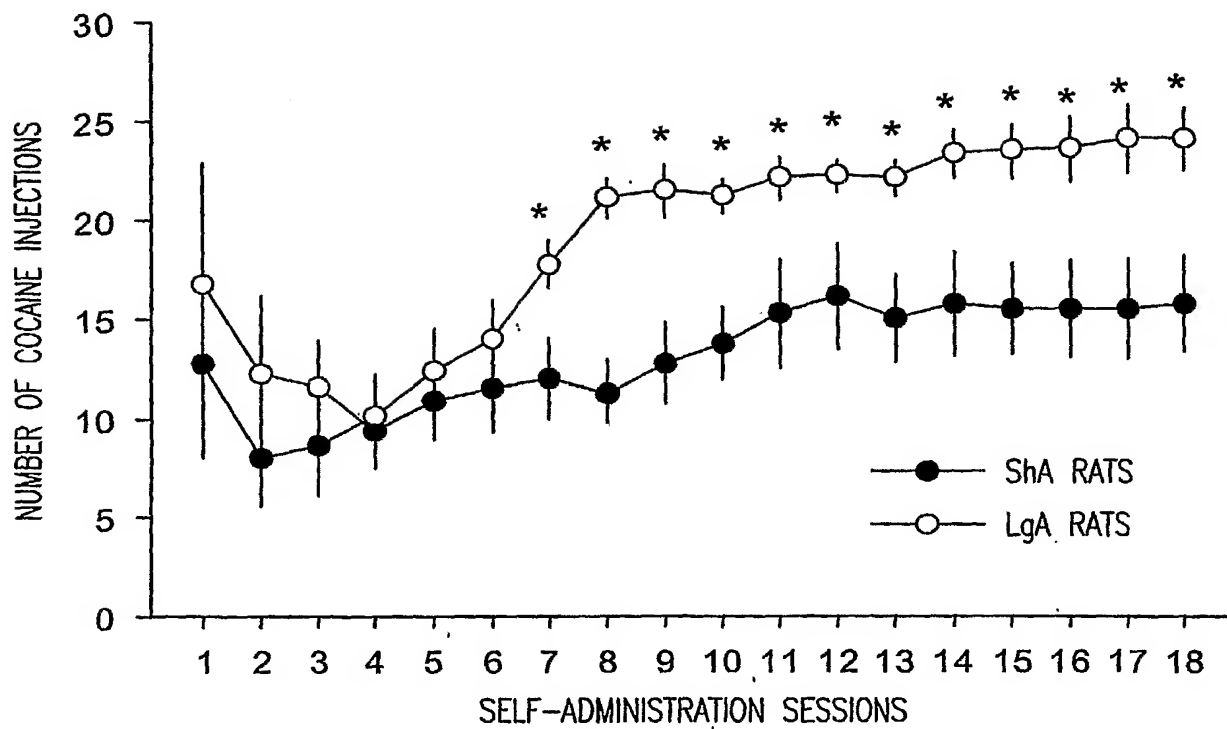


FIG. 1A

2/4

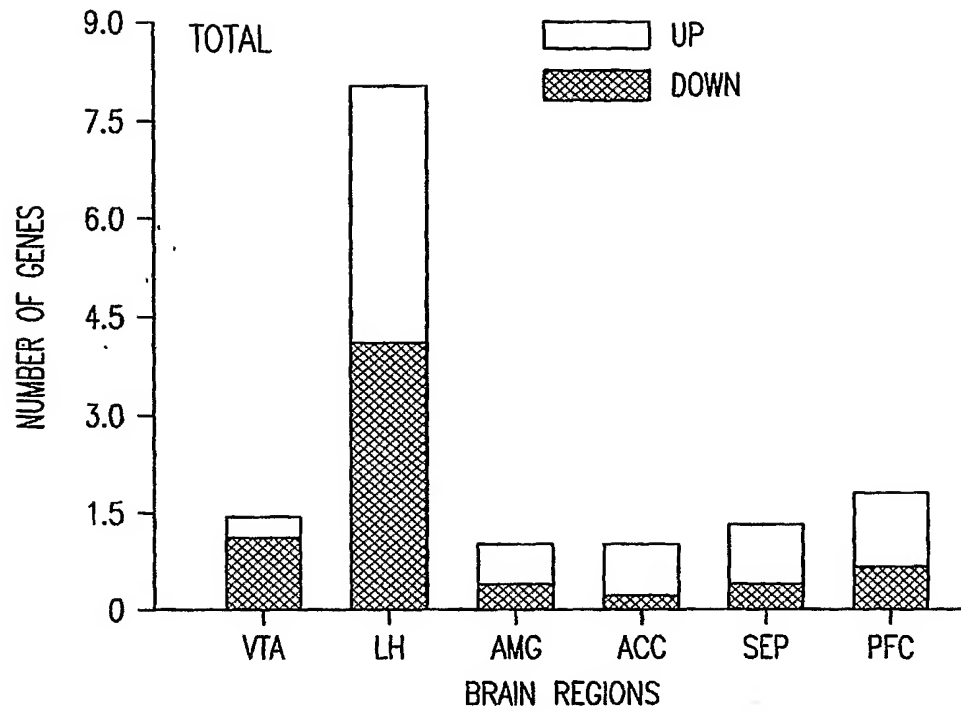


FIG. 1B

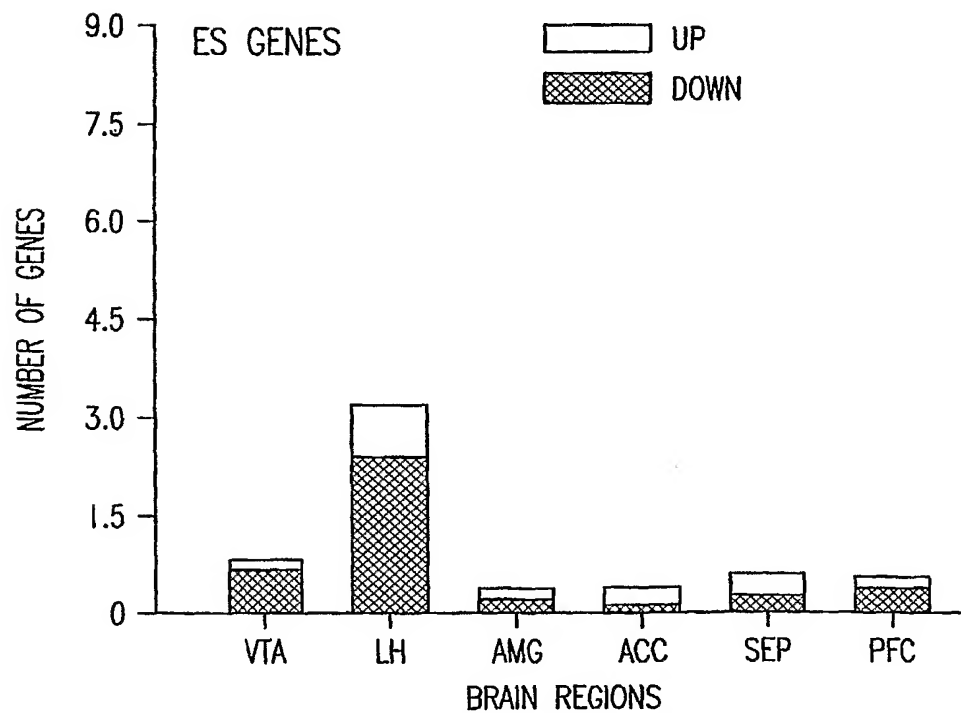


FIG. 1C

3/4

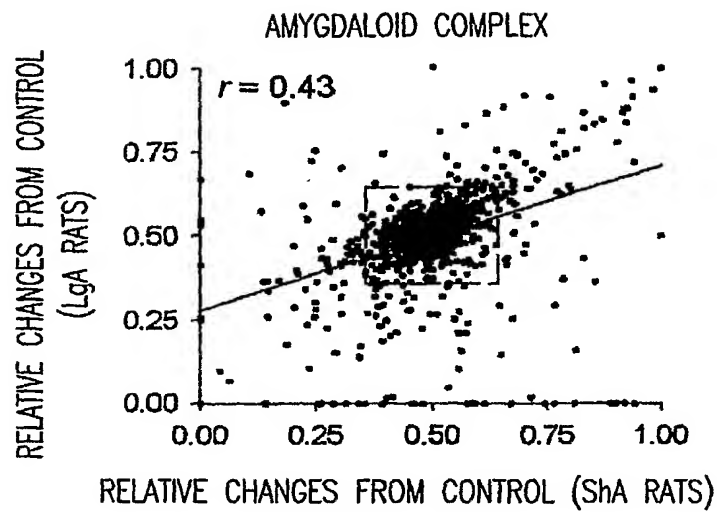


FIG. 2A

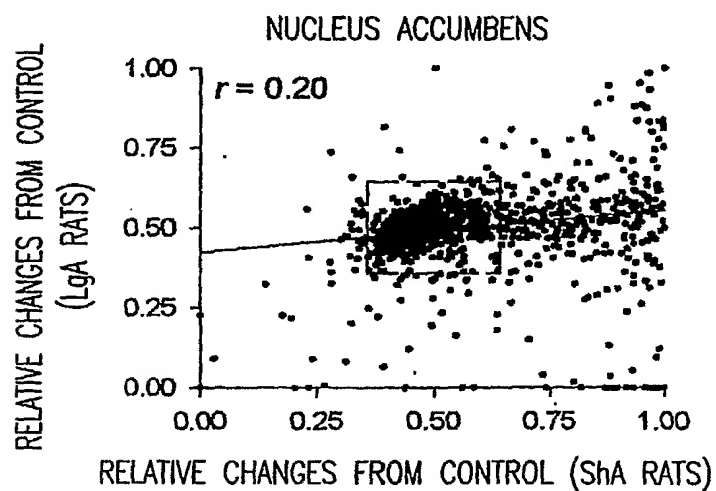


FIG. 2B

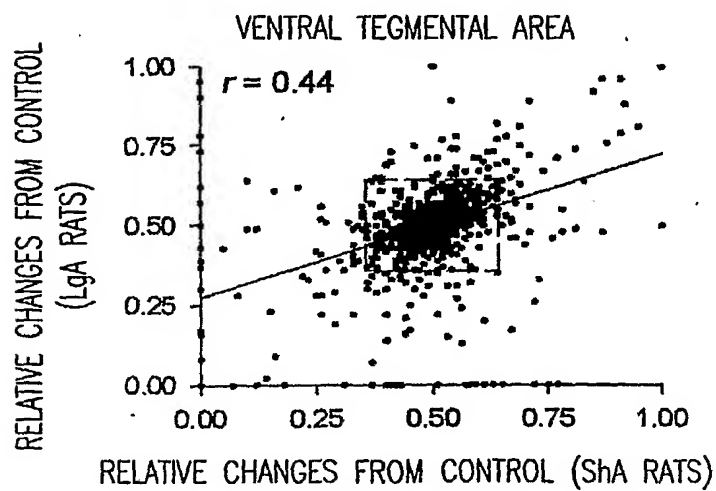


FIG. 2C

4/4

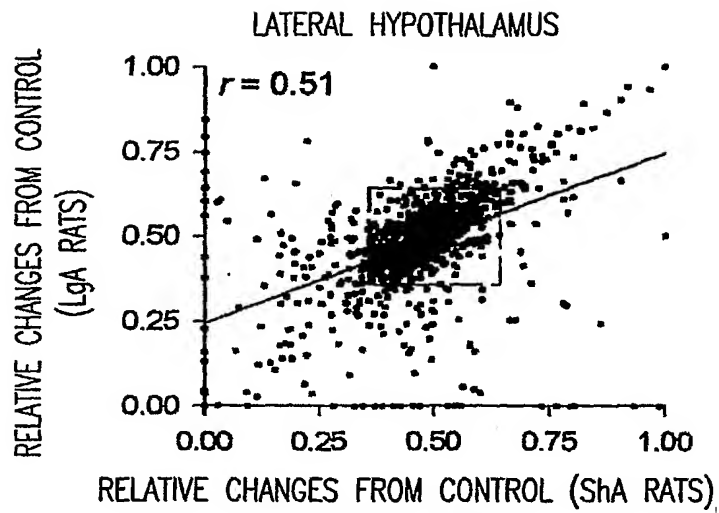


FIG. 2D

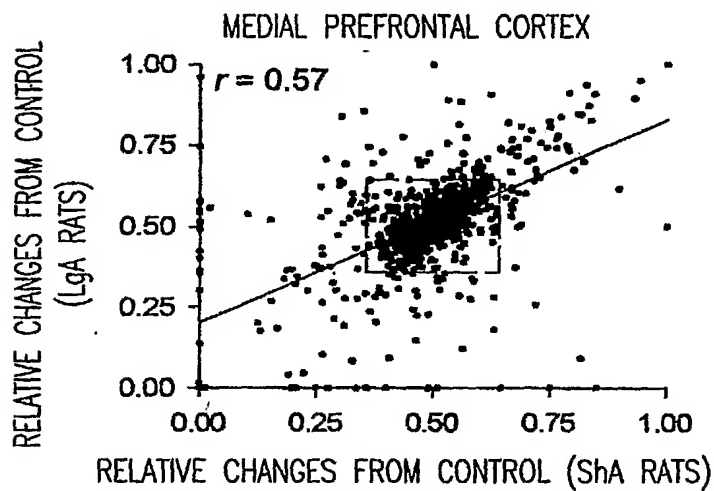


FIG. 2E

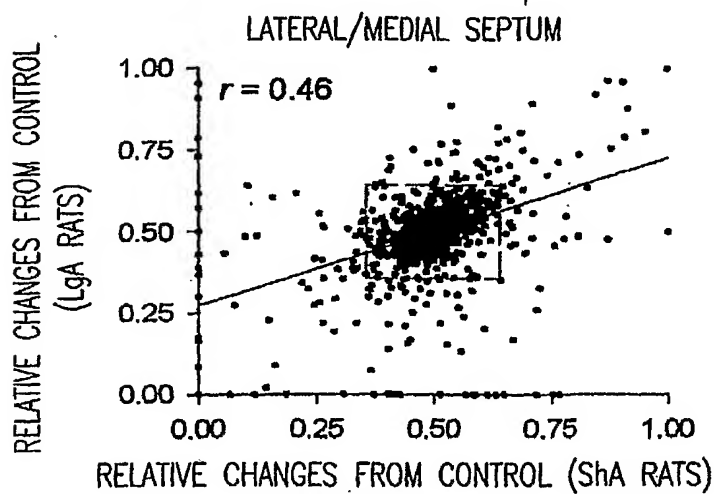


FIG. 2E

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
24 June 2004 (24.06.2004)

PCT

(10) International Publication Number
WO 2004/053099 A3

(51) International Patent Classification⁷: **A01N 61/00**,
37/18, 43/04, A61K 31/00, 38/00, 31/70, 39/395, C12Q
1/00, 1/68, G01N 33/53, 33/567

(21) International Application Number:
PCT/US2003/039499

(22) International Filing Date:
10 December 2003 (10.12.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/432,496 10 December 2002 (10.12.2002) US

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE,
GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR,
KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK,
MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT,
RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR,
TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (BW, GH,
GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE,
SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA,
GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments

(88) Date of publication of the international search report:
29 December 2005

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: A METHOD FOR TREATMENT OF DRUG ADDICTION AND FOR SCREENING OF PHARMACEUTICAL AGENTS THEREFOR

(57) Abstract: The present invention is directed to a method for treatment of drug addiction and screening methods for identifying pharmaceutical agents that ameliorate or prevent the deleterious effects of addiction. The invention is as well directed to a group of genes and a group of gene products that are up or down regulated as a result of addiction.



WO 2004/053099 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/39499

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A01N 61/00, 37/18, 43/04; A61K 31/00, 38/00, 31/70, 39/395; C12Q 1/00, 1/68; G01N 33/53, 33/567
US CL : 514/1, 2, 44; 424/130.1; 435/4, 6, 7.1, 7.2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/1, 2, 44; 424/130.1; 435/4, 6, 7.1, 7.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MEDLINE, BIOSIS, EMBASE, CAPLUS, USPATFULL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DE VRIES et al. Neural systems underlying opiate addiction. J. Neuroscience. 01 May 2002, Vol. 22, No. 9, pages 3321-3325.	1-12
A	LEE et al. Opioid receptor polymorphisms and opioid abuse. Pharmacogenomics. 2002, Vol. 3, No. 2, pages 219-227.	1-12
A	KREBK et al. Pharmacotherapy of addictions. Nat Rev Drug Discovery. September 2002, Vol. 1, pages 710-726	1-12
A	BHARGAVA, H. Opioid peptides, receptors, and immune function. NIDA Res Monogr. 1990, Vol. 96, pages 220-233.	1-12

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Date of the actual completion of the international search

19 October 2005 (19.10.2005)

Name and mailing address of the ISA/US

Mel Stop PCT, Attn: ISA/US
Commissioner of Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Facsimile No. (703) 305-3230

Date of mailing of the international search report

07 NOV 2005

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